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A CYTOLOGICAL STUDY OF ARTIFICIAL PARTHENOGENESIS IN CUMINGIA

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FOUR TEXT FIGURES AND EIGHT PLATES

I. INTRODUCTION

The following experiments with the egg of the mollusc Cumingia tellinoides were begun at Woods Hole in the summer of 1914. At first it was intended simply to find an easy method of inducing artificial parthenogenesis and to make a cytological study of eggs undergoing such development. A method was soon found which gave a fair percentage of cleavage and a few swimming larvae in most experiments. Early in the course of the experiments, however, it was observed that while some eggs gave off both polar bodies in the normal manner, others formed only one, and still others passed at once to a 2-cell stage without forming polar bodies at all. From the cytological study made in the following winter, it was found that in some of the eggs subjected to the treatment, both nuclei formed by the division of chromosomes in the first polar spindle are retained in the egg, and that these two nuclei fuse. It seemed as if a sort of self-fertilization had taken place in these eggs, and the question whether this process was the beginning of normal development became the central one of the problem. The later experiments were, therefore, made for the purpose of finding out whether the swimming larvae obtained by the parthenogenetic treatment came from eggs which had formed polar bodies or from those which had not. When it was found that normal larvae do, in fact, come from eggs in which maturation has been suppressed, it was still necessary to determine from a further study of preserved material whether the eggs in which the polar nucleus fused with the egg nucleus were those which

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developed to larvae. There might, of course, be some other way in which eggs without polar bodies could develop, and those in which the fusion of nuclei took place might prove to be, after all, incapable of further development. Finally, besides settling this question, it was thought that a study of the preserved material would throw light on the question as to which of the two maturation divisions was the true reducing division.

II. METHODS

a. Experimental. Cumingia is fairly common in the waters around Woods Hole, and each female, when ripe, yields a large number of eggs. The animals are brought in dry, and do not begin to spawn until placed in sea-water. It is therefore easy to obtain eggs free from contamination by sperm, by placing each individual in a separate dish of water. To guard further against contamination by sperm the usual precautions with regard to clean dishes were observed. Controls of all the experiments were kept and carefully watched. Very little tendency towards parthenogenetic development such as Morgan ('10) observed in the course of his experiments on Cumingia was seen in these controls. In one case a few eggs were found which had formed the first polar body, but no cleavage stages or larvae were found in this or in any other control.

The agents used to obtain parthenogenetic development in these experiments were heat and hypertonic sea-water. Heat alone, hypertonic sea-water alone, and hypertonic sea-water followed by heat gave some development, but the results were not so satisfactory as when heat was used first and followed by hypertonic sea-water. This is, of course, in accord with Loeb's method of artificial parthenogenesis, in which a cytolytic agent is followed by a 'corrective.' It does not come within the scope of the present study to say whether the effect of the hypertonic sea-water is in fact corrective as Loeb thinks, or additive as R. S. Lillie has maintained more recently. Loeb and Wasteneys induced normal segmentation and formation of larvae in the eggs of Cumingia by sensitizing them with $SrCl_2$ and treating

them with ox-serum followed by hypertonic sea-water. No cytological study of these eggs was made, and from the brief notes the authors have published it is impossible to say whether the results of this treatment correspond with those set forth in the following pages.

The technique of these experiments is simple. A small flask of sea-water is suspended in a beaker of water and warmed over a flame till the temperature of the water in the flask is slightly above that to which one wishes to expose the eggs. The introduction of a little cool sea-water with the eggs lowers the temperature somewhat, and it is an easy matter to keep it constant for an hour or more within the rather wide limits necessary for these experiments.

The temperatures to which the eggs were subjected varied from 32°C. to 37°C., and the length of the exposures from $1\frac{1}{2}$ to 90 minutes. The treatment with hypertonic sea-water was also varied, and the interval between the two treatments, as well as the interval between the spawning of the eggs and the beginning of the experiment. A detailed study of these variations is given in a later section and in the tables.

Some eggs were fertilized, in order that a comparison might be made between the normal and the parthenogenetic development. In fertilizing eggs, care must be taken to avoid polyspermy by using a dilute sperm suspension.

b. Microscopic methods. Material was taken from the experimental cultures at varying intervals, and preserved for cytological study, and corresponding series were made of the normally fertilized eggs. Almost all of this material was preserved in Bouin's fluid. A few sets were fixed in Mme. Danchakoff's modification of Zenker's fluid, in which 8–10 per cent formalin is substituted for the glacial acetic acid.

As the eggs are very small, they were stained in tôto before embedding. Conklin's picro-hematoxylin was found to be most useful for this purpose. Some of the material was stained with borax carmine, but this makes the sections opaque and practically valueless unless they are bleached with chlorine gas.

The eggs were carried up through xylol to paraffin in the vials in which they were preserved, and were kept in the vials while the process of enfiltration took place in the embedding oven. When they were ready for embedding each vial was taken from the oven and held against a cool surface till the paraffin at the bottom of it began to solidify. The rest of the paraffin was then poured off, and the solid mass at the bottom of the vial was picked out with a fine forceps and embedded in a dish of hot paraffin, just as if it were a piece of tissue. As all the eggs settle to the bottom of the vial during the process of enfiltration, the solid mass contains them all and none are lost. If the thing is done quickly so that the mass of paraffin is still very soft, there is no difficulty in getting it out of the vial or in embedding it.

On account of the large number of eggs cut in a single section it was often difficult to follow one egg through a series of sections. For this reason, large numbers of paramecia were fixed and embedded with the eggs, and their presence served as a guide to the location of an egg in successive sections. Sections were cut 7–9 m. in thickness and stained with iron hematoxylin. Some were counterstained with Orange-G, but this is no improvement on the plain iron hematoxylin stain.

III. DEVELOPMENT OF FERTILIZED EGGS

The maturation and fertilization of the egg of Cumingia have already been described by Jordan. For the sake of convenience, however, it is best to review the phenomena here, especially as the question of the individuality of the chromosomes and the genetic continuity of the centrosomes which were Jordan's main interest are not those which concern us.

The egg is laid after the first polar spindle has been formed. This spindle (represented in figure 1) is large, and lies near the center of the egg. It might, in fact, be mistaken for a cleavage spindle in the metaphase, if it were not for the form of the chromosomes which is entirely different in the two divisions. If the eggs are not fertilized or subjected to any parthenogenetic agent they remain in this condition, with the spindle near the middle. After fertilization has taken place, however, the

spindle moves nearer to the periphery of the egg, the eighteen chromosomes divide, and a normal anaphase ensues (fig. 3). The cytoplasm of the egg pushes out to form the first polar body, and the first maturation mitosis is completed (fig. 4).

The second maturation division follows without the intervention of a resting stage (fig. 5). During the formation of the second polar body, the first is often pulled back into a concavity of the surface of the egg, so that it would be invisible in a surface view, but the walls between the two cells remain intact. Such a condition is represented in figure 7, but in figure 6 we see a very different case in which the first polar body and the cytoplasmic bud for the second both stand out prominently beyond the surface of the egg. The second polar spindle (represented in figures 6, 7, and 9) is rather smaller than the first, but is like it in having eighteen chromosomes which divide and go to the poles of the spindle in a perfectly normal manner (fig. 9).

The chromosomes in the two divisions are, however, quite different in form. Those of the first maturation are varied in shape and size, but are all larger than those of the second polar spindle. No regular series can be made of them, as has been done with the chromosomes of some other forms. The two clearest equatorial plates of this spindle that were found are represented in figures 2a and 2b. They are alike in having two rings and one U-shaped chromosome apiece, but one could not say whether these forms are constant without much more study of the point than seems worth while in the present connection. The remaining fourteen chromosomes of the first polar spindle are round, oval, or cross-shaped bodies of various sizes. At the beginning of the anaphase, as soon as the arrangement in a regular plate is lost, the chromosomes undergo a marked reduction in size. The daughter-chromosomes of the anaphase (fig. 3) seem less than half as large as the chromosomes of the equatorial plate.

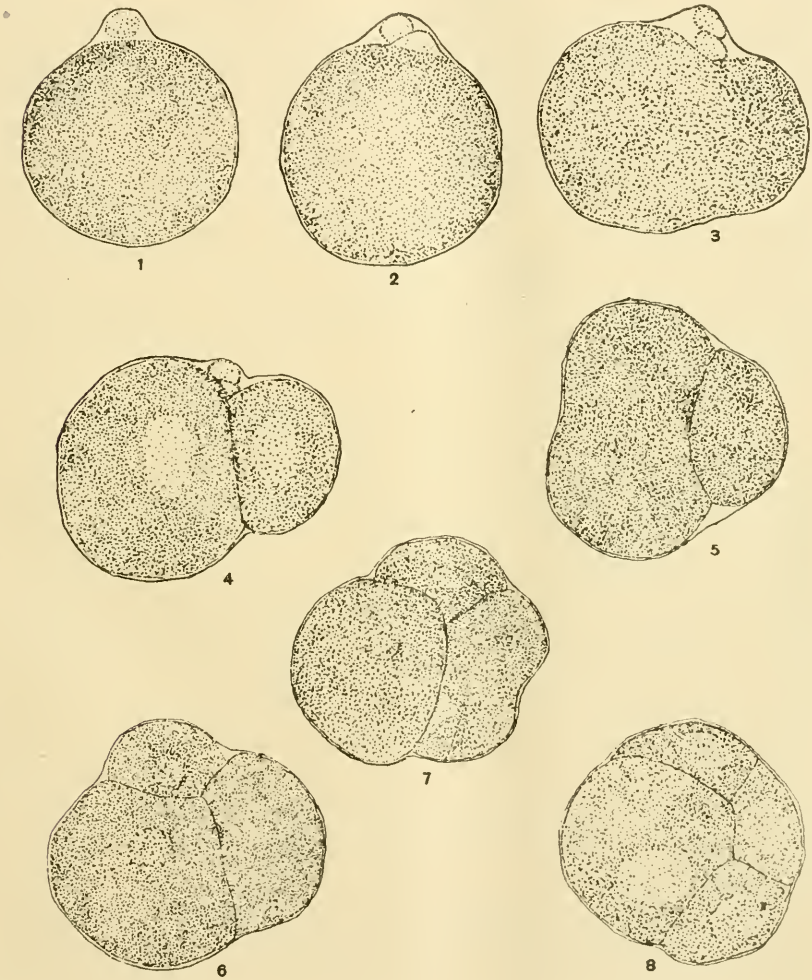
The equatorial plate of the second polar spindle is represented in figure 8. Here the chromosomes are all in the form of short rods with somewhat irregular outlines. They are much more constant in size and shape than those of the first polar spindle.

After the formation of the second polar body, the chromosomes that have remained in the egg form a large female pronucleus. The sperm nucleus has enlarged, in the meantime, and the two nuclei unite (figs. 10 and 11). No attempt has been made to trace the history of the aster which appears before the two nuclei fuse.

The first cleavage spindle does not lie in the center of the egg, as the first division is an unequal one. It is about the size of the first polar spindle, but the chromosomes are very different in form as well as in number from those of the maturation divisions. In the cleavage spindles they are long, thin rods, or threads, with slight terminal swellings. They are so much bent and intertwined in the equatorial plate that an accurate count is impossible, but the number is presumably thirty-six. Figure 13 shows the anaphase of the first cleavage, in which the chromosomes are small rods. Throughout the early cleavage the chromosomes have, in the metaphase, the form of long threads; but in the later development a gradual change of shape is seen. Figure 15 shows chromosomes from a middle cleavage stage, and figure 16 is from an egg fixed nine hours after fertilization. Here the chromosomes have been reduced to the short rods which Jordan says are characteristic of Cumingia, and which are considerably smaller than the chromosomes of the fourth cleavage, for instance, shown in figure 14.

The normal cleavage pattern of Cumingia has been described by Browne ('10) in her study of the effect of pressure. It is illustrated here for comparison with the cleavage of parthenogenetic eggs in the text-figure 1 (surface views) and figures 17 to 28 (sections). The first cleavage plane passes through the polar bodies and divides the egg into two unequal blastomeres (fig. 17, text-fig. I, *3* and *4*). Of these the larger one (lettered *CD* in the drawings) is usually the first to divide. The spindle forms in the middle of the cell, but moves to an eccentric position, with the outer end slanted towards the cell *AB*, while still in the metaphase (figs. 19 and 20). The result of this division is a 3-cell stage in which the cells are all unequal in size, *C* being equal to about half of *AB* (figs. 23 and 24), text-fig. I, *6* and *7*).

The division of *AB* which follows leads to the 4-cell stage represented in figure 25 and in text-figure I, *8*, in which there are three cells, *A*, *B*, and *C*, of about the same size and a larger cell *D*. The size-relations of these cells are very constant in normally fertilized eggs. The time-relations of the divisions are less constant, as may be seen from the different phases of the spindles in figures 18 to 24. In some cases *AB* and *CD*



divide simultaneously, as in figure 22, so that the egg passes at once from a 2-cell to a 4-cell stage.

The normal cleavage is not given here in detail beyond the 4-cell stage. A 5-cell stage (not illustrated) results from the division already begun in the egg illustrated in figure 25. The cell d_1 which is cut off from D is about the size of A, B or C, so that there are four equal cells forming a cap on the cell D which is still larger than any of them. Four hours after fertilization the egg is in the condition shown in figure 26. The large cell is a descendant of D, the smaller ones of A, B, C, and d_1. Figure 27 shows a late cleavage stage in which the lineage of the cells cannot be traced.

The 24-hour larva is sketched in surface view in text-figure 2, *1*. The larvae are lively swimmers at this time, and it is difficult to get a drawing of a living specimen which shows more than the general size and shape. The section (Fig. 28) shows, however, that the mid-gut, stomodeum, and shell-gland are already formed, and that scattered mesenchyme cells are present in the segmentation cavity.

IV. DETAILED STUDY OF EXPERIMENTS

a. Best method of inducing artificial parthenogenesis. As has been said, many variations were made in the treatment by which artificial parthenogenesis was induced. The most significant variations are those in the exposure to heat, and the experiments may therefore be grouped according to the temperature to which the eggs were exposed. Variations in the hypertonicity of the sea-water used as a second treatment and in the length of the exposure to this agent seem to be of comparatively slight importance, and it makes little difference in the result whether the eggs are used as soon as they are shed or allowed to stand for some time, first. The length of the interval between the heating and the treatment with hypertonic sea-water is also unimportant.

For counting the percentage of eggs that develop to larvae the following method was used: Five hundred eggs were picked out at random from the culture and set aside, and the number of

larvae that developed from them was counted the next day. If there were no larvae in this lot, but some in the main dish, the number is recorded as 'few.' If there were only a very few in the main dish, that fact is recorded.

Although the eggs vary greatly in their susceptibility to treatment, a tabulation of the experiments shows that the highest percentages of larvae result from an exposure of 60 minutes to a temperature of 32–33°C. This treatment was used in eighteen experiments, of which four failed to produce any larvae and three gave less than 0.2 per cent. Of the remaining eleven experiments, ten yielded larvae in proportions varying from 0.2 per cent to 4 per cent and in one 18 per cent of the eggs developed to this stage. The unusually successful result of this last-mentioned experiment is an illustration of the variation in the susceptibility of the eggs to the treatment. All attempts to obtain a similar result by repeating the experiment exactly were failures—no one of them gave more than 4 per cent of larvae. One might think that the experiment was contaminated by sperm except for the fact that the control showed no development, and there was no evidence of fertilization among the eggs preserved from the experiment for cytological study.

Although 60 minutes is the optimum length of exposure to 32–33°C., larvae may be obtained by shorter exposures. Even a 30-minute treatment may give 0.2 per cent larvae, and a 45-minute one may give 1 per cent. Exposures lasting as long as 90 minutes also give fair results. To summarize, we may say that out of forty experiments in which this temperature was used, six gave no larvae at all, nine gave less than 0.2 per cent, and the remaining twenty-five gave 0.2 per cent or over. For further details of these experiments, the reader is referred to table 1.

The next group of experiments is made up of those in which the eggs were heated to 33–35°C. For this temperature the optimum exposure is 30 minutes. This group includes four experiments which were made at the beginning of the study, when no counts were made to obtain the percentage of larvae. Setting these four aside, however, we have the following re-

TABLE 1[1]

Heat 32–33°C.

NO. OF EX.	CONDITION OF EGGS	EXPOSURE TO HEAT	INTERVAL	HYPERTONIC SEA-WATER	POLAR BODIES	CLEAVAGE	LARVAE
		min.	*min.*			*per cent*	*per cent*
54B	Fresh	30	15	KCl5 + SW50 9 min.	A good many	25	0.2
56A	Fresh	30	5	KCl5 + SW50 40 min.	Many (1st and 2d)	8	Few
77A	Stood 2 hours	45	4	KCl5 + SW50 60 min.	21%	20	Very few
63B	Fresh	45	19	KCl10 + SW50 30 min.	3.5%	Poor	None
63A	Fresh	45	3	KCl10 + SW50 32 min.	10.5%	Poor	1
71	Stood 2½ hours	50	3	KCl10 + SW50 30 min.	Some	6	0.6
68	Fresh	55	4	KCl10 + SW50 29 min.	Some	10	1
$49A_1$	Fresh	60	0	KCl5 + SW50 13 min.	Some 1st, few 2d	19	0.4
$49A_2$	Fresh	60	15	KCl5 + SW50 13 min.	Few	17	0.4
56B	Fresh	60	5	KCl5 + SW50 40 min.	Some	27	2.2
59A	Fresh	60	2	KCl5 + SW50 60 min.		20	3
77B	Stood 2 hours	60	5	KCl5 + SW50 60 min.	9%	14	0.5
59B	Fresh	60	2	KCl5 + SW50 1½ hrs.		23	1.4
59C	Fresh	60	2	KCl5 + SW50 2 hrs.		16	1
$48A_1$	Fresh	60	35	KCl10 + SW50 5 min.	Some 1st, no 2d	13	Few
62	Fresh	60	2	KCl10 + SW50 30 min.	1.3%	Poor	0.6
58	Fresh	60	2	KCl10 + SW50 30 min.	Very few	16	4.2
65A	Fresh	60	2	KCl10 + SW50 30 min.	25% ±	5	0.2
65B	Stood 3 hours	60	5	KCl10 + SW50 30 min.	Very few	26	18
70A	Stood 2 hours	60	3	KCl10 + SW50 30 min.	5%	30	None
66	Stood 2 hours	60	4	KCl10 + SW50 30 min.	10%	14	None
75A	Stood 3 hours	60	4	KCl10 + SW50 30 min.		Poor	Very few
75B	Stood 3½ hours	60	5	KCl10 + SW50 30 min.		Poor	Very few

[1] Throughout the experiments a 2½ M solution of KCl or NaCl was used in making up the hypertonic seawater.

72A	Fresh	60	6	KCl10 + SW50 30 min.	None?	15	None
74	Stood $2\frac{1}{2}$ hours	60	5	KCl10 + SW50 35 min.	Few		None
$52A_1$	Fresh	70	15	KCl5 + SW50 10 min.	Few	10	Very few
$52B_1$	Fresh	70	16	NaCl5 + SW50 10 min.	Few	16	Very few
$49B_1$	Fresh	70	0	KCl5 + SW50 13 min.	None	25	1
$49B_2$	Fresh	70	15	KCl5 + SW50 13 min.	None	23	1.8
$52A_2$	Fresh	70	15	KCl5 + SW50 20 min.	Few	13	Few
$52B_2$	Fresh	70	16	NaCl5 + SW50 20 min.	None	13	0.4
$52A_3$	Fresh	70	15	KCl5 + SW50 30 min.	Few	10	0.2
$52B_3$	Fresh	70	16	NaCl5 + SW50 30 min.	None	12	0.2
$48B_1$	Fresh	70	35	KC10 + SW50 5 min.	Some (first)	13	None
55A	Fresh	75	4	KCl5 + SW50 30 min.	Few	28	0.8
55B	Fresh	75	4	KCl5 + SW50 40 min.	Few	28	1
55C	Fresh	75	4	KCl5 + SW50 50 min.	Few	32	1.6
55D	Fresh	75	4	KCl5 + SW50 60 min.	Few	25	1.8
$48C_1$	Fresh	80	35	KCl10 + SW50 5 min.		11	Few
56C	Fresh	90	5	KCl5 + SW50 40 min.	Some	18	1.2

sults from these experiments, of which there are twenty-two. Eight gave no larvae at all, twelve gave few, or very few, and only two gave over 0.2 per cent. The highest percentage obtained was 4 per cent. These experiments are set forth in detail in table 2.

Finally, a few experiments were made with a temperature of 35–37°C. If the eggs are subjected to this temperature for ten minutes, fragmentation ensues. The optimum length of exposure is two minutes, but there was only one of the eleven experiments with this temperature which resulted in the formation of larvae, and in this case they were very few. Table 3 shows the results of these experiments. A few experiments were made with temperatures below 32°C. but they were entirely unsuccessful.

b. Experiments to show that larvae come from eggs which have not formed polar bodies. Up to this point, no mention has been made of the effect that different exposures to heat have on polar body formation. We must consider this question, as it gives the first evidence that it is eggs which have not formed polar bodies that develop to larvae. It has been seen that exposing the eggs to 32–33°C. for an hour gives the highest percentage of larvae, while the two-minute exposure to 36–37°C. gives very few larvae, or none at all. It is, however, the latter group of experiments which gives the highest percentage of polar body formation. Compare, for instance, No. 53 (table 3) and No. 65 B (table 1). In the first, polar bodies were formed in almost all the eggs, but only 2 per cent divided, and none developed to larvae. In the second, the polar bodies were very few, while 26 per cent of the eggs divided and 18 per cent grew to larvae. These experiments represent the extremes, and perhaps the point is more fairly illustrated by an experiment in which one lot of eggs was divided into two portions. One half was exposed to a temperature of 36–37°C. for two minutes, the others kept at 32–33°C. for thirty minutes. The first half showed 22 per cent with polar bodies, hardly any cleavage, and no larvae; the second had 10 per cent polar body formation, 25 per cent dividing and 0.2 per cent larvae.

TABLE 2

Heat 33–35°C.

NO. OF EX.	CONDITION OF EGGS	EXPOSURE TO HEAT	INTERVAL	HYPERTONIC SEA-WATER	POLAR BODIES	CLEAVAGE	LARVAE
		min.	*min.*			*per cent*	*per cent*
57A	Fresh	10	2	KCl5 + SW50 60 min.	Many (1st and 2d)	7	Few
60A	Fresh	10	12	KCl10 + SW50 15 min.	Some	7	Few
87	Fresh	25	2	KCl10 + SW50 32 min.	Some	Poor	Very few
85	Fresh	30	7	KCl5 + SW50 30 min.	Some	11	None
88A	Fresh	30	2	KCl5 + SW50 30 min.	Some	18	4
89A	Fresh	30	5	KCl5 + SW50 30 min.	Some		Few
88B	Fresh	30	2	KCl5 + SW50 60 min.	Some	22	0.5
89B	Fresh	30	5	KCl5 + SW50 60 min.	Some		Few
57B	Fresh	30	3	KCl5 + SW50 60 min.	Some	19	Few
86	Fresh	30	12	KCl5 + SW50 2 hrs.	No development—Fragmentation		
17B	Fresh	30	3	KCl8 + SW50 8 min.	Some	Some	Many
20	Fresh	30	0	KCl8 + SW50 8 min.	Many	Some	Few
35	Fresh	30	0	KCl8 + SW50 8 min.	First and second	Few	Few
36	Fresh	30	0	KCl8 + SW50 8 min.		Fair	None
41	Fresh	30	0	KCl8 + SW50 8 min.	Some	Good	None
60B	Fresh	30	0	KCl10 + SW50 15 min.	None?	10	None
83A	Fresh	30	9	KCl10 + SW50 30 min.	Some	Poor	Few
83B	Fresh	30	30	KCl10 + SW50 30 min.	Some	Poor	None
76	Stood ½ hr.	30	5	KCl10 + SW50 30 min.	Some	13	None
84A	Fresh	38	7	KCl5 + SW50 32 min.	Few	55	Few
84B	Fresh	38	7	KCl5 + SW50 60 min.		47	Few
84C	Fresh	38	7	KCl5 + SW50 90 min.		43	Few
84D	Fresh	38	7	KCl5 + SW50 129 min.		28	Few
57C	Fresh	45	2	KCl5 + SW50 60 min.	None?	16	Few
57D	Fresh	60	1	KCl5 + SW50 60 min.	None?	9	Few
80	Stood 1¼ hr.	60	8	KCl5 + SW50 60 min.	Hardly any	15	None

TABLE 3

Heat 35–37°C.

NO. OF EX.	CONDITION OF EGGS	EXPOSURE TO HEAT	INTERVAL	HYPERTONIC SEA-WATER	POLAR BODIES	CLEAVAGE	LARVAE
		min.	*min.*			*per cent*	*per cent*
70B	Stood $2\frac{3}{4}$ hours	$1\frac{1}{2}$	$3\frac{1}{2}$	KCl10 + SW50 30 min.	25%	Less than 1%	None
53	Fresh	2	30	KCl5 + SW50 10 min.	Almost all eggs	2%	None
54A	Fresh	2	15	KCl5 + SW50 15 min.	Many	Hardly any	None
$46A_1$	Fresh	2	40	KCl8 + SW50 10 min.	Many (1st and 2d)	11%	Very few
72B	Stood $1\frac{1}{4}$ hours	2	7	KCl10 + SW50 15 min.	Some	3%	None
67	Stood 1 hour	2	4	KCl10 + SW50 30 min.	Some	None	None
73	Stood $1\frac{1}{2}$ hour	3	47	KCl10 + SW50 16 min.	Some	Poor	None
69	Stood $2\frac{1}{2}$ hours	4	18	KCl10 + SW50 31 min.	Some	Poor	None
$46B_1$	Fresh	5	40	KCl8 + SW50 10 min.	Some	None	None
46C	Fresh	10	Large proportion of eggs fragmented—No development.				

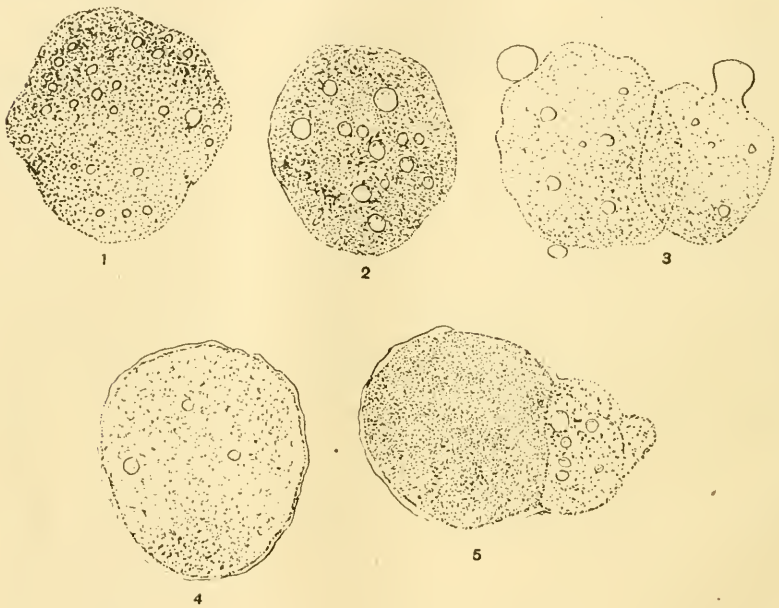
In most experiments, no attempt was made to count the percentage of maturation, as the polar bodies would be hidden behind the egg in many cases. In the experiment just described, the counts were made to give the relative numbers in the two parts of the experiment. In one half 22 per cent of the eggs had polar bodies that were in such a position that they could be seen without rolling the eggs around; in the other half the count was only 10 per cent. The percentage of cleavage, on the other hand, represents the actual proportion of the eggs that are undergoing segmentation, as a 2-cell stage is recognizable as such from any point of view.

These experiments serve as an illustration to show that, in general, in a case where polar body formation is good, cleavage is poor and few larvae develop. But they do not prove conclusively that larvae come from eggs in which maturation has been suppressed. To do this, it was necessary to resort to the method of isolating eggs without polar bodies and observing their development. The highest powers of the binocular microscope were used in selecting the eggs, and each one picked out was first rolled around with a fine brush and viewed from all sides to make sure that it had, in fact, no polar bodies. In all, 1215 such eggs were isolated; and from these, five larvae developed. One of these larvae is shown in text-figure 2, *2*. It is smaller than the larva from the normally fertilized egg, but is evidently cellular, and is fairly normal in external appearance.

This showed, then, that larvae could develop from eggs which had not formed polar bodies, but did not prove that eggs which had undergone maturation could not also form larvae. That such eggs could divide at least once was shown from the 2-cell stages with polar bodies which were occasionally found in the cultures. 313 eggs with one or two polar bodies were isolated, and from these a single swimmer developed. This was not, however, a normal larva. It is illustrated in text-figure 2, *3*. Evidently it has developed without further cleavage from a 2-cell stage.

Abnormal swimmers like this are not uncommon in the experiments. Sometimes an egg will be found that has been

differentiated entirely without cytoplasmic cleavage, such as is shown in text-figure 2, *4*, or one like text-figure 2, *5*, in which one cell has developed to a ciliated swimming structure while the other remains an inert mass. This phenomenon of differentiation without cleavage is known in the parthenogenetic development of other forms. In the egg of Chætopterus, for instance, Lillie ('02) has found it to be of common occurrence, and Scott ('06) has also produced it in the eggs of Amphitrite.



In both of these cases it appears to be independent of maturation. Eggs without polar bodies may develop in this way as well as those which have matured fully. In Cumingia there is no evidence that the phenomenon is confined to eggs with polar bodies. The preserved material does not give a complete series of stages, so that the cytology of this kind of development could not be worked out at this time.

Table 4 gives the details of the experiments in which eggs without polar bodies were separated from those which had undergone maturation. The numbers are not very large, to be

sure, but one may fairly conclude from them that eggs in which maturation has been suppressed are able to develop to cellular larvae; while those in which it has been completed form abnormal non-cellular swimmers if they develop at all. This conclusion is supported by the experiments already described, which showed that a high percentage of maturation resulted in a low percentage of cleavage-stages and larvae. It is also supported by the cytological evidence given in the following sections.

TABLE 4

Isolated eggs

	1 OR 2 POLAR BODIES	2 POLAR BODIES	NO POLAR BODIES	AT RANDOM
Number isolated	100			
Number of larvae	0			
Number isolated	4		296	300
Number of larvae	0		2	0
Number isolated	34		179	200
Number of larvae	0		1	2
Number isolated	25		90	200
Number of larvae	0		0	0
Number isolated	15			300
Number of larvae	0			3
Number isolated	25		100	400
Number of larvae	0		0	0
Number isolated	25		100	400
Number of larvae	0		0	2
Number isolated	40		100	300
Number of larvae	1		0	2
Number isolated	4		100	300
Number of larvae	0		1	0
Number isolated			100	200
Number of larvae			0	1
Number isolated	5	7	100	400
Number of larvae	20	0	1	16
Number isolated	9		50	
Number of larvae	0		0	
Total number isolated	306	7	1215	3000
Total number of larvae	1	0	5	26

V. CYTOLOGICAL STUDY OF EGGS WITHOUT POLAR BODIES

a. Fusion of first polar nucleus with egg nucleus. When the eggs are subjected to heat, the first polar spindle moves from its central position towards the periphery of the egg, as it does when the egg is fertilized. In experiments in which the eggs were heated to 34–35°C. the spindle is often found to be contracted, as is shown in figure 49. It may be so much shortened that the chromosomes are in a compact mass in which the form of individuals cannot be distinguished. In other cases, however, especially in the experiments in which a lower temperature was used, the spindle is entirely normal in this stage.

The form of the chromosomes of the first polar spindle in eggs which are undergoing parthenogenetic development is normal. Figure 50 illustrates an equatorial plate from a parthenogenetic egg, and though the rings and the loop which were found in some normal plates (figs. 2a and 2b) are not represented here, the general size and form of the chromosomes are alike in the two plates. The rings are to be seen, moreover, in the spindle represented in figure 49, and it is possible that their absence from the plate of figure 50 is due simply to insufficient extraction of the stain.

In the stages following the metaphase the behavior of the chromosomes is entirely normal, but a complete nuclear division takes place without any corresponding cytoplasmic division. Figure 51 shows clearly the beginning of the division of the chromosomes. Some have already separated and are grouped at the poles of the spindle; a few are just splitting. The same reduction in the size of the chromosomes that occurs in the fertilized eggs at the beginning of the anaphase of this first maturation division, is to be seen here. In the next figure (52) a slightly later stage is represented in which all the chromosomes have divided, and in figure 53 the migration to the poles of the spindle is almost finished. The splitting of the chromosomes is complete, as is shown in figures 52 and 53. If any lag in the center of the spindle, they are daughter-chromosomes. Figure 53 represents a stage of the parthenogenetic egg corre-

sponding to that of the fertilized one shown in figure 3. More chromosomes are drawn in figure 53 than in the corresponding figure of the normal egg, owing to the fact that in the latter case the section did not contain the whole of the spindle, but as far as the form and size of the chromosomes go the two are alike. The important difference is that, while the outer pole of the spindle in the fertilized egg lies in a cytoplasmic bud, there is no sign of any such bud in the parthenogenetic egg. In this, as in the later stages, the whole spindle lies within the circumference of the egg.

The transformation of the anaphase groups of chromosomes into resting nuclei is like the telophase of any division except for the absence of cytoplasmic cleavage. The chromosomes break up into a mass of chromatin at each pole of the spindle, as is shown in figure 54. Here we have also a third mass, half-way between the poles, which was doubtless formed from a few lagging chromosomes. Then follows the formation of numerous small vesicles and the gradual fading of the achromatic figure (fig. 55). The small vesicles fuse and enlarge (fig. 56) till finally two resting nuclei are formed such as are shown in figure 57. Sometimes a third small vesicle is found, half-way between the two large ones. This represents, of course, chromosomes which have not gone to either pole of the spindle (fig. 54) but such a vesicle always fuses sooner or later with one of the large ones.

The nuclei thus formed enlarge considerably, come to lie close together, and eventually fuse (fig. 58, 59 and 60). The stages resemble very closely the fusion of the pronuclei in a normally fertilized egg, except for the fact that no aster is present in them (cf. figs. 58 to 60 with figs. 10 and 11). The nuclei which represent the first polar body and the secondary oöcyte nucleus are about the size of the male and female pronuclei, and the product of their fusion looks much like a normal cleavage nucleus. It is, of course, like the normal cleavage nucleus in being the result of the fusion of two nuclei, each of which contained the haploid number of chromosomes. The egg is fertilized by the first polar body instead of by the sperm, and contains the full amount of chromatin that was present in the primary oöcyte.

This process of fusion of the first polar nucleus and the egg nucleus is characteristic of the experiments from which larvae develop, and the stages described above are of common occurrence. There are, moreover, no evidences of any other method of development in eggs which have no polar bodies. Up to a late stage of the development of the cultures, eggs are found in the preserved material in which the first polar spindle in metaphase still occupies the center of the egg. These have evidently not responded at all to the parthenogenetic treatment, and they go to pieces without having advanced beyond this condition.

b. Cleavage and development to larvae. The preparations for the first cleavage in the eggs without polar bodies are not abnormal except for the absence of the asters, which do not appear till late in the cleavage and are never very large. In the stage represented in figure 61 the chromatic material has begun to condense, in irregular masses, and later (fig. 62) definite threads are to be seen.

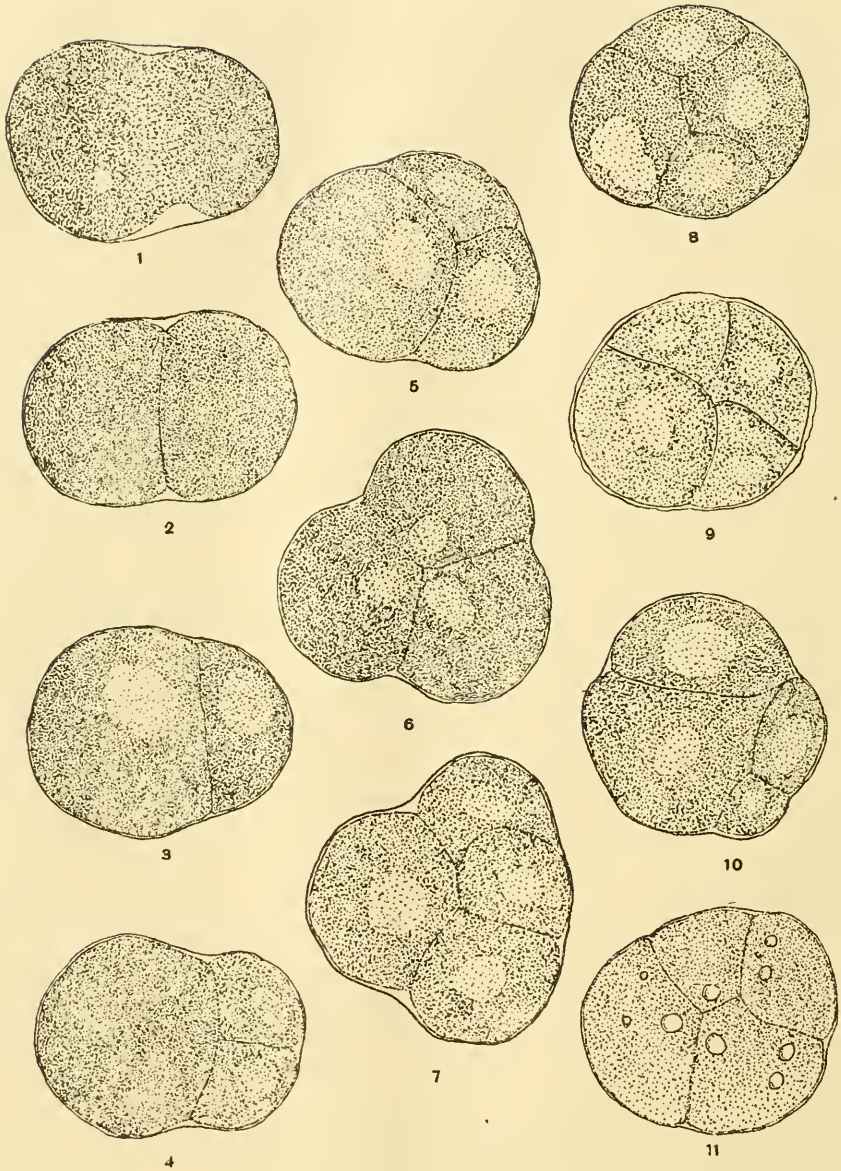
One would naturally expect to find thirty-six chromosomes in the equatorial plate of the first cleavage of one of these eggs, since thirty-six daughter-chromosomes went to make up the cleavage nucleus. When the plates are examined, however, they are found to contain fifty or sixty chromosomes apiece. These have not the form characteristic of the chromosomes of the first cleavages of the fertilized eggs. Instead of being threads, they are compact rods, not much more than half the size of the normal cleavage chromosomes (figs. 63 to 65). They are, in fact, much more like the rods found in the late cleavages of the normal egg (cf. fig. 16). In the metaphase of the first cleavage the chromosomes form a plate or a ring and there is little difficulty in counting them. The number is not constant: of the three plates represented in figures 63, 64 and 65 one has fifty-six, another fifty-eight and the third sixty-one chromosomes. It seems probable that there has been an abnormal distribution of the chromatic material into fifty-five or sixty small rods instead of thirty-six threads. Were the number of rods seventy-two one might suppose the daughter-chromosomes of the first polar spindle to have been bivalent, but there seems to

be no significant relation between the number actually found and the haploid or diploid number of the species. The facts are in accord with the idea that Brauer ('94) had from his study of Artemia salina that the number of chromosomes is of no importance in the development of the organism provided the mass of chromatic material is undiminished.

The achromatic portion of the spindle is very faint in the metaphase of the first cleavage. Figure 64 shows a side view of one of the rings of chromosomes, with a faint aster but no spindle. In the anaphase the figure is better developed and the chromosomes are small round bodies (fig. 66). In later cleavages the large number of chromosomes persists and the size is only slightly reduced. Figure 67 shows a plate of fifty-two chromosomes from the larger cell of a 2-cell stage; figure 68, the chromosomes from a middle cleavage stage.

The eggs undergoing parthenogenetic development often follow the normal cleavage pattern so closely that they could not be distinguished from fertilized eggs. Text-figure 3 shows surface views of a number of early cleavage stages of parthenogenetic eggs. Such examples as numbers *1*, *3*, *5*, *8* and *9* are not uncommon in the experiments. Here, as far as one can tell, cleavage is proceeding in a perfectly normal manner. In other cases, the size-relations of the cells have been modified. Often the first two blastomeres are approximately equal in size (text-fig. 3, *2*) and sometimes a 3-cell stage is found composed of equal cells (text-fig. 3, *6*). Corresponding abnormalities are seen in the 4-cell stage. On the whole, however, the surface views show an approximation to the normal cleavage pattern which is rather unusual in eggs developing by artificial parthenogenesis.

In the sections of these early cleavages illustrated in figures 29 to 35, the normal relations are shown in many cases, and of course the same variations from the normal pattern that were found in surface views are repeated here. Figure 30, for instance, shows a 2-cell stage in which the blastomeres are so nearly equal that it was impossible to letter them to correspond to the cells of a fertilized egg. All these drawings are made from slides in which it was possible to trace an egg through all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its sections, so as to be sure that it had no polar bodies. Abnormalities in the positions of the spindles are sometimes found. For instance, in figure 31 the first cleavage has divided the eggs into the unequal cells *AB* and *CD* as usual, but the outer pole of the spindle formed in *CD* for the next cleavage slants away from *AB* instead of towards it. If the figure were reversed, so that the slant of the spindle were normal (cf. fig. 20), its position would be abnormal, for it would then lie at the vegetative pole of the egg instead of at its animal pole. There may also be variations in the order of the divisions of the cells, as is shown in figure 33 where *D* has a spindle developed in a somewhat abnormal position before *AB* has begun to divide at all. Cleavage in the parthenogenetic eggs is considerably slower than in the fertilized ones, so that one finds 3- and 4-cell stages in which all the nuclei are in the resting condition. Such stages are not often found in the fertilized eggs where the cleavages succeed each other more rapidly.

After the 4-cell stage, the cleavage pattern becomes somewhat confused. The 5-cell stage represented in figure 35, for instance, is not at all like the 5-cell stage of the normal egg. This is not illustrated here, but has been described as a cap of four small cells on top of the large cell. The division goes on, apparently without any disturbance of the nucleo-plasma relation, the nuclei being entirely normal in their appearance. No stage was found to correspond to figure 26. Figure 36 shows a parthenogenetic egg of the same age as the normal one drawn in figure 27. The difference in the size of the cells is undoubtedly due to the slower development of the parthenogenetic egg.

The surface view of the parthenogenetic larva from an egg without polar bodies has already been described, and is shown in text-figure 2, *2*. The section of the larva (fig. 28) shows it to be solid, or nearly so, and composed of cells which, though larger than those of a larva from a fertilized egg, are normal in their appearance. There are no organs to be made out, such as are seen in the section of a normal larva of this age.

Besides these normal cleavage stages, one finds in the preserved material evidence of a marked tendency towards nuclear

division without cytoplasmic cleavage. Often there is a 3- or a 4-cell stage which is normal except for the presence of two nuclei in one of the cells. More abnormal cases are shown in figures 38 to 41, where cytoplasmic cleavage is abnormal or entirely lacking. I have not found stages of mitotic division in the multinucleate cells, and am inclined to believe that the nuclei arise by a process of fragmentation. Their irregular arrangement and variable size seems to indicate an entirely abnormal condition. Whether it might lead to differentiation and the formation of unicellular swimmers is not clear. Sometimes the nuclei are arranged peripherally in later stages (fig. 41) and have a fairly normal appearance. These multinucleate cells are found for the most part in experiments in which cleavage was poor and no larvae were formed.

VI. CYTOLOGICAL STUDY OF EGGS WHICH HAVE FORMED POLAR BODIES

The amount of material preserved from experiments in which a large percentage of eggs formed polar bodies is unfortunately not very great, so that the study of the cytology of these eggs is by no means complete. There are many gaps in the series which must be filled in at some other time. The first stage to be noted in the formation of the first polar body is the early anaphase represented in figure 69. This is a perfectly normal anaphase like that of the first polar spindle in the fertilized egg. The cytoplasmic bud is, perhaps, abnormally large, and there seems to be a tendency for this first polar body to have more cytoplasm in the parthenogenetic than in the fertilized egg. The chromosomes, however, behave in a normal way.

The later stages of this division and the formation of the second polar spindle are unfortunately lacking in the material I have preserved. The next stage to be found is the metaphase of the second maturation. In the case represented in figure 70, the spindle lies unusually far from the periphery of the egg, but instances have been found in which it occupied a normal position, close beneath the first polar body. The chromosomes of this spindle are like those of the second maturation of the fer-

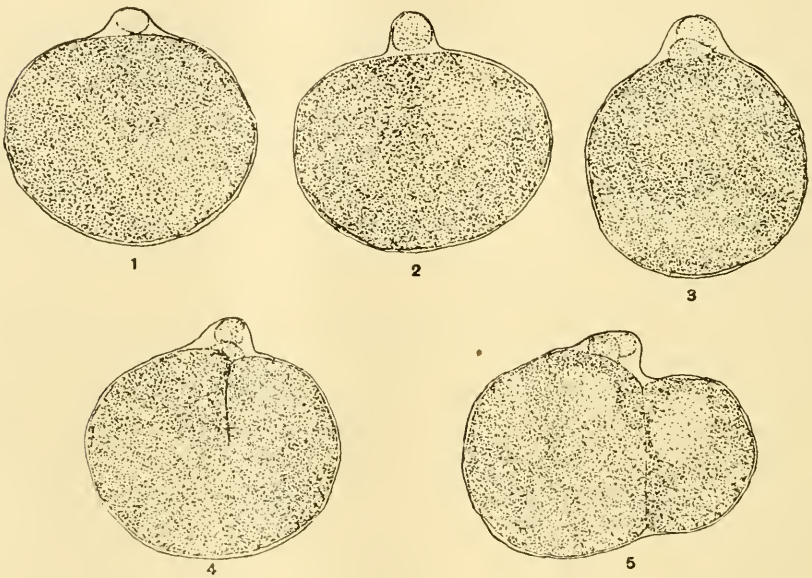
tilized egg, as far as can be told from a side view of the spindle (cf. figs. 7 and 70). Up to this point, then, maturation has proceeded in these eggs in a manner closely approximating the normal course of events. From here on, however, we must trace separately the fate of eggs which form only one polar body and that of those in which maturation is completed.

a. Eggs with one polar body. In some eggs, a suppression of the second polar body may take place, similar to the suppression of the first polar body which has been described. The steps are apparently the same. The spindle lies well below the surface of the egg, and there is no attempt to form a polar body. The chromosomes divide, however, and go to the poles of the spindle, where they form vesicles, just as the chromosomes of the first polar spindle have been seen to do in other cases (figs. 71 and 72). The vesicles are, however, decidedly smaller than those formed by the chromosomes of the first polar spindle. Figure 73 shows a stage which corresponds to that of figure 56, and illustrates this difference.

The series of stages showing the fusion of the two resting nuclei formed by the suppression of the second polar body is by no means complete. Two small vesicles are sometimes found close together, apparently about to fuse, as is shown in figure 74. Also, one finds single vesicles, much larger, as represented in figure 75. It seems most probable that two resting nuclei are formed from the daughter-chromosomes of this second maturation spindle, and that the nuclei fuse; but a positive statement can not be made until more material has been preserved and studied.

Only two cases of normal cleavage of eggs with one polar body were found in the preserved material. Of these, one is an anaphase of the first cleavage—unfortunately too late a stage to show anything of the form and number of the chromosomes (fig. 42). The spindle is normal in appearance, but lies farther from the animal pole than that of a normal egg. A 2-cell stage was also found which had only one polar body. Here, the cleavage plane did not pass through the polar body, but the egg was otherwise normal in appearance.

Besides these two examples of cleavage there are cases to be found of abnormal division in a plane at right angles to the normal first cleavage plane. One hardly knows whether to call the cell cut off in this manner a large polar body or a small blastomere. The position of the masses of chromatin and the nuclei lead one to think the division may have resulted directly from the second polar spindle. Figure 44 shows one of these cases, in which the cell cut off is of such a size that it might be



called a polar body. In figure 46, on the other hand, if the division has been completed the cells would have had about the size-relations of *AB* and *CD* in a normal egg. Figure 45 illustrates an intermediate condition.

b. Eggs with two polar bodies. No stages illustrating the formation of the second polar body were found in the preserved material, but there is no reason to suppose that this process is abnormal in the parthenogenetic eggs in which it takes place. Surface views of the maturation are shown in text-figure 4, and a 2-cell stage with two polar bodies. In these nothing appears

abnormal except the size of the first polar body in *2* and *3*. The first polar body quite often divides, so that three are found (figs. 47 and 76). The vesicles that are formed in the egg after the extrusion of the second polar body are normal and fuse to form a single nucleus (figs. 76 and 77). This nucleus is decidedly smaller than that formed by the fusion of vesicles from either the first or the second polar spindle, but it is possible that in the case figured it had not attained its full size. One could not draw any conclusions from the size of the nuclei with so few examples on which to base them.

In the first cleavage of eggs with two polar bodies, one finds a plate of eighteen chromosomes. These have very nearly the form and size of the cleavage chromosomes in the normally fertilized eggs, the condition being entirely different from that of eggs in which maturation has been suppressed (cf. fig. 78 with fig. 63 and with fig. 74).

Cleavage stages of eggs with two polar bodies are rare. Figure 47 illustrates a 2-cell stage, and figure 48 the only 4-cell stage with two polar bodies that was found in the material. The possibility that an occasional one of these eggs with one or two polar bodies may develop is, of course, not absolutely excluded. I have found no evidence of later development among them, but do not consider the study of these eggs complete.

VII. REVIEW OF LITERATURE AND DISCUSSION

The connection between maturation and parthenogenetic development is one which has proved to be of particular interest in many cases of natural parthenogenesis. Sometimes, of course, the parthenogenetic egg forms both polar bodies in a normal manner. In the bee, for instance (Petrunkevitch '01), maturation is completed in the drone egg and development begins with the reduced number of chromosomes. Petrunkevitch says that the diploid number is restored by a division of the chromosomes unaccompanied by cytoplasmic cleavage. According to Meves ('07), no such regulation takes place, and the drone has the haploid number of chromosomes throughout its development. In spermatogenesis, Meves says, there is no

further reduction of chromosomes, the single spermatocyte division being homotypic, so that the haploid number appears again in the spermatids. Doncaster ('06) found that in the parthenogenetic egg of the sawfly both polar bodies are formed and the same is true of the eggs of the parasitic hymenoptera investigated by Silvestri ('06, '08, '15). In all of these cases the polar nuclei are retained in the cytoplasm of the egg, and the second fuses with one or both of the daughter-nuclei resulting from a division of the first. The 'copulation nucleus' thus formed does not, however, fuse with the egg nucleus. Sometimes it takes part in the formation of an envelope surrounding the embryo—sometimes it degenerates. (See Silvestri.)

In some other naturally parthenogenetic forms, however, there is no reduction of the chromosomes of the egg before development begins. For instance, the eggs of the pædogenetic larva of Miastor undergo only one maturation division (Hegner, '14 a). This is true also of the parthenogenetic eggs of Aphis rosae and Aphis oenotherae, in which only one polar body is formed regardless of the sex of the individuals developing from the eggs (Stevens, '04).

In some forms, the suppression of the true reducing division is associated with the determination of the sex of the embryo developing from the parthenogenetic egg, as Whitney ('08) has shown to be the case with Hydatina senta. Here the females come from eggs which have formed two polar bodies, and the males from those which have formed but one. An essentially similar case is that of the gallfly, in which some of the parthenogenetic eggs form two polar bodies and the rest none at all. The ones which have undergone the maturation divisions develop into male flies with the haploid number of chromosomes; the others have the diploid number and become females (Doncaster, '10, '11). In the Phylloxerans (Morgan, '09) neither the male-producing nor the female-producing parthenogenetic egg forms two polar bodies, but the single maturation division reduces the number of chromosomes in one and not in the other. Two accessory chromosomes are thrown out in the maturation of the male-producing egg, while the female egg has the full

number throughout its development. The essential point in all these cases is of course the same; namely, that the females develop from eggs in which reduction has not occurred.

Finally, there are two cases of natural parthenogenesis described in which there is apparently a fertilization of the egg by a polar nucleus—a condition similar to that induced in the eggs of Cumingia by the treatment described above. The first of these instances is that of Astropecten. Natural parthenogenesis had been observed in the eggs of this starfish by Greeff in 1876 and the cytological phenomena were studied by O. Hertwig in 1890. In this egg the first polar body is given off, and the second polar spindle formed normally. But instead of being extruded, the second polar nucleus is formed inside the egg and fuses with the female pronucleus. The segmentation of such eggs is irregular, seldom leading to the formation of normal blastulae. Some eggs were found which had formed two polar bodies, but these seemed incapable of further development.

Brauer's work ('94) on Artemia salina shows a similar condition. In the parthenogenetic eggs of this crustacean, the first polar body is given off, but the second is not. Development may proceed, according to Brauer, in one of two ways. A cleavage spindle may be formed at once, or the second polar spindle may form and give rise to two nuclei which are both retained in the egg, and fuse to form a cleavage nucleus. In the first case, the egg develops with 84 chromosomes, in the second with 168. Brauer assumes that the 84 chromosomes were bivalents and concludes that "für die Einleitung einer normalen Entwickelung eines Thieres die Zahl der Chromosomen gleichgültig ist wenn nür die Masse dieselbe ist." Here, also, when both polar bodies are formed, there is no normal development. It may be mentioned that although this work of Brauer's has been widely accepted and quoted, Hertwig's on Atropecten is really a more convincing instance of self-fertilization. Petrunkevitch ('02) has pointed out the lack of intermediate stages in Brauer's series between the formation of the second polar spindle and the fusion of the two nuclei, and has stated his belief that the binucleate condition is a pathological one, not lead-

ing to normal development. Although Hertwig's series of stages is not complete, it is more nearly so than Brauer's.

Up the present time there has never been any definite proof that in artificial parthenogenesis development depended on the retention of polar bodies or the restoration in some way of the full number of chromosomes. In the eggs of the sea-urchin, which are among the most favorable objects for parthenogenetic study, fairly normal plutei are obtained from eggs which contain the haploid number of chromosomes. Here the reduction divisions are completed before the egg is subjected to parthenogenetic treatment, and there is no later regulation of the number of chromosomes.

Some experimental studies have been made of eggs which are laid before the maturation divisions begin, with the object of finding out whether they would respond to treatment better in this stage than after the polar bodies have been formed. In working with the starfish, Asterias glacialis, Delage ('01) obtained the best results by applying the treatment during the anaphase of the first polar spindle. From this and from the fact that the second polar body is not formed in the majority of the eggs that develop, he concluded that the essential factor was the retention of chromatin. Garbowski ('03) also worked on Asterias glacialis, but according to his results, eggs with two polar bodies develop rather better than those with only one or none at all, though all three kinds may develop. Later, Delage ('04) modified his idea, stating that the reason the stimulus must be applied during the maturation period was that the egg responds more readily when it is in the process of karyokinetic division than in the resting stage.

In many other cases of artificial parthenogenesis, it seems to make no difference whether the polar bodies are formed or not. In Thalassema, for instance, (Lefevre, '07) eggs which have formed polar bodies develop as well as those which have not. In this form the first polar division may be suppressed, the spindle giving rise to two nuclei which fuse to form a cleavage nucleus. There is evidence that a second division of each of the two nuclei may take place before they fuse, giving a stage with

four nuclei. If the first polar body is formed in the normal manner, the second polar spindle may form two nuclei which fuse. Lefevre finds, however, that there is no difference in the course of development, whether the eggs are fertilized by the first or by the second polar nucleus or obliged to develop with the haploid number of chromosomes.

The results obtained by Allyn ('12) in her study of artificial parthenogenesis in Chaetopterus seem to be of the same nature; though one cannot be sure, because of the small amount of material she had for cytological study. There is evidence that the first or the second polar body may be retained in the egg and that the cleavage spindles have the diploid number of chromosomes; but it is not shown conclusively whether this is a necessary preliminary to development or not.

Kostanecki's study ('04, '11) of parthenogenesis in Mactra is interesting as the only other cytological study of parthenogenesis in molluscs. Here, as in the cases already mentioned, one or both polar bodies may be formed, or the egg may omit maturation entirely. No self-fertilization was observed here. The eggs developed to swimming larvae, often by a process of repeated nuclear divisions followed by a simultaneous cleavage into a number of small cells.

In all of these cases, then, the formation or suppression of polar bodies seems to be a matter of indifference. They are hardly to be compared with the development of naturally parthenogenetic eggs, for the cleavages are irregular, and the whole condition evidently pathological. In the sea urchin, indeed, where both maturation divisions occur, development is fairly normal. Artificial parthenogenesis in this form might be compared to natural parthenogenesis in the hymenoptera in which both polar bodies are formed. The phenomena in Cumingia, on the other hand are comparable to the cases of natural parthenogenesis in which one polar body is suppressed. To make the comparison exact, one must suppose reduction to occur normally with the formation of the first polar body in Cumingia. This seems a warrantable assumption when we consider the relation of the two maturation divisions to parthenogenetic development.

If the first polar nucleus fuses with the egg nucleus, cleavage follows. If the second polar nucleus fuses with the egg nucleus, there is no more normal development than if the second polar body is formed and its chromatin rejected. It is clear that it is the second maturation division, not the first, that is the mass reduction.

The case of Cumingia is, then, essentially like that of Astropecten. In both cases, the qualitative division of the maturation is suppressed, and the polar nucleus reunites with the egg nucleus. It is interesting to note that Astropecten is not a form in which there is a regularly recurring parthenogenetic generation, but one in which development without fertilization is abnormal. Artemia is the only form in which parthenogenesis occurs naturally where there is any indication of self-fertilization. In other animals which have a regular parthenogenetic generation the reducing division is not begun at all, but the egg passes at once from the mass division of the chromatin to cleavage. It has already been pointed out that the eggs of Cumingia in which division of the chromosomes of the first polar spindle does not occur are incapable of development. Artificial parthenogenesis in this mollusc, like natural parthenogenesis in Astropecten, is halfway between the usual conditions of artificial and natural parthenogenesis. In the former, development is often independent of maturation; in many cases of the latter the retention of the full amount of chromatin, without any attempt at the qualitative division, insures development.

In conclusion, I should like to express my thanks to Professor Petrunkevitch for his invaluable assistance in the working out of this problem.

VIII. SUMMARY

1. The eggs of Cumingia can be made to develop parthenogenetically by exposing them to temperatures ranging from 32°–37°C. and then exposing them to hypertonic sea-water.

2. The highest percentages of cleavage-stages and swimming larvae are obtained when the eggs are exposed to 32°C. for an

hour. The highest percentage of polar body formation is obtained when they are exposed to 37°C. for $1\frac{1}{2}$ minutes.

3. By isolating eggs without polar bodies and observing their development, it is found that they may form fairly normal swimming larvae. Those with polar bodies form non-cellular swimmers, if they develop at all.

4. Cytological study of eggs which have not formed polar bodies shows that the chromosomes of the first polar spindle divide, and two nuclei are formed which fuse to form a cleavage nucleus.

5. In the cleavage of these eggs there are fifty or sixty small rods of chromatin, instead of the thirty-six threads found in the normal egg. Cleavage often follows the normal pattern very closely.

6. The chromosomes of the second polar spindle may also divide and form two nuclei which fuse. Very few of the eggs with one polar body divide.

7. Eggs with two polar bodies may, in rare instances, divide once or twice. Eighteen chromosomes (the haploid number) are found in such eggs.

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PLATE 1

EXPLANATION OF FIGURES

All figures on this plate from normally fertilized eggs.

1 Metaphase, first polar spindle.

2a and 2b Equatorial plates, first polar spindle.

3. Anaphase, first polar spindle.

4 Telophase, first polar spindle.

5 Completion of first maturation division.

6 Metaphase, second polar spindle.

7 Metaphase, second polar spindle.

8 Equatorial plate, second polar spindle.

9 Anaphase, second polar spindle.

Magnification, 2600 diameters.

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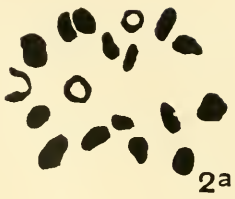
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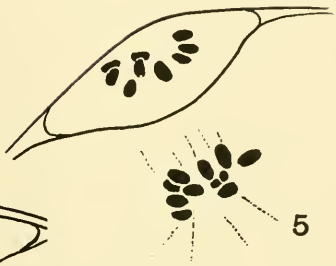
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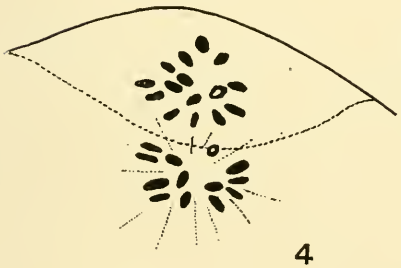
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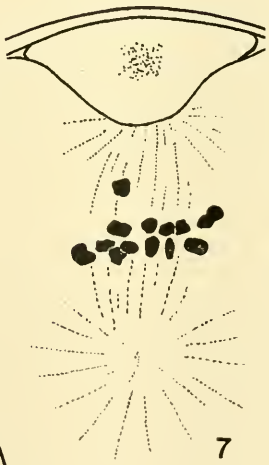
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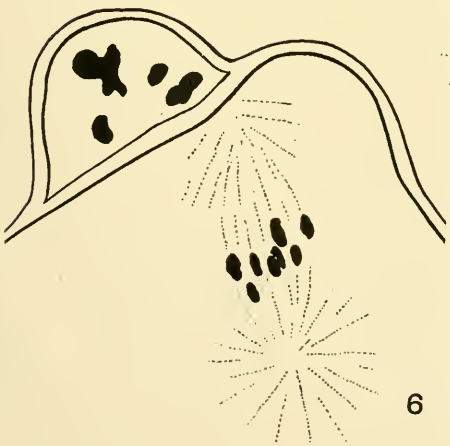
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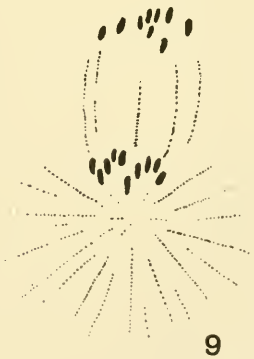
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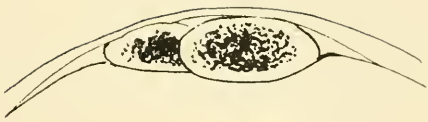
PLATE 2

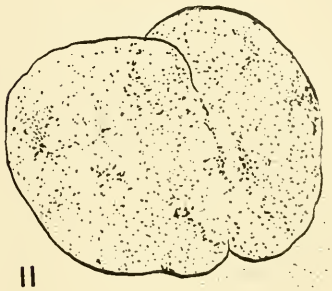
EXPLANATION OF FIGURES

All the figures on this plate are from normally fertilized eggs.

10 Approach of male and female pronuclei.
11 Fusion of male and female pronuclei.
12 Metaphase, first cleavage spindle.
13 Anaphase, first cleavage spindle.
14 Chromosomes from the fourth cleavage.
15 Chromosomes from middle cleavage stage.
16 Chromosomes from an egg fixed nine hours after fertilization.

Magnification, 2600 diameters.





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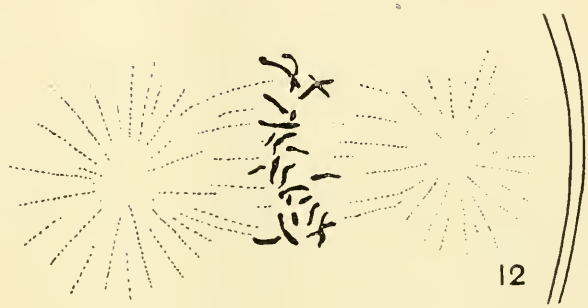

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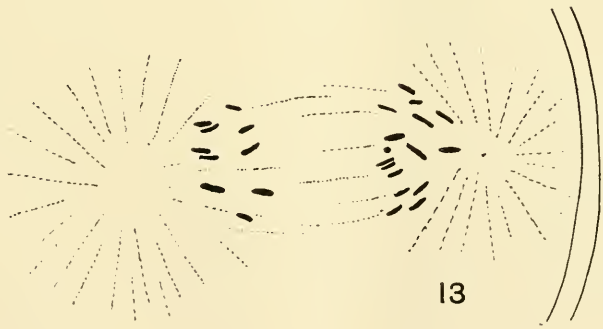

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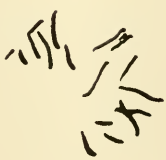

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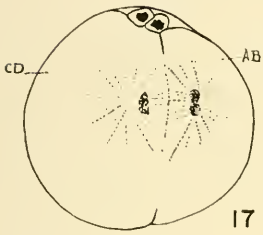
PLATE 3

EXPLANATION OF FIGURES

17 to 28 From normally fertilized eggs.
17 Telophase, first cleavage.
18 Two-cell stage.
19 to 21 Formation of three-cell stage.
22 Formation of four-cell stage directly from two-cell stage.
23 and 24 Three-cell stage.
25 Four-cell stage.
26 Middle cleavage stage.
27 Nine hours after fertilization.
28 Twenty-four hours after fertilization.
29 to 31 From parthenogenetic eggs.
29 Telophase, first cleavage.
30 and 31 Two-cell stage.
Magnification, 575 diameters.



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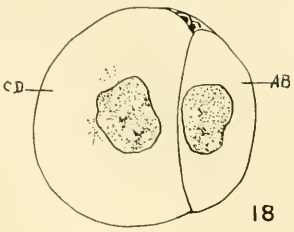

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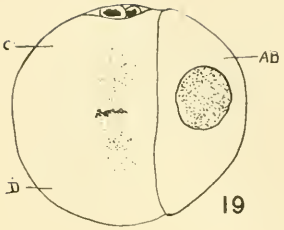

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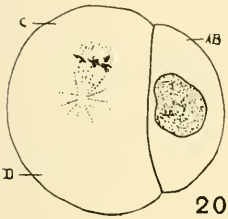

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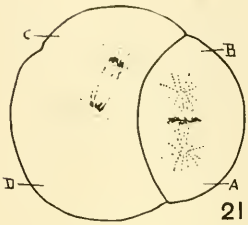

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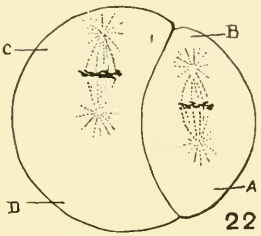

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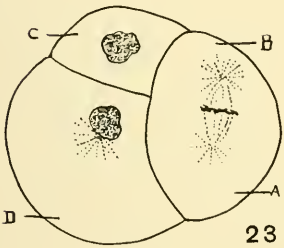

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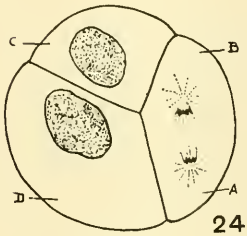

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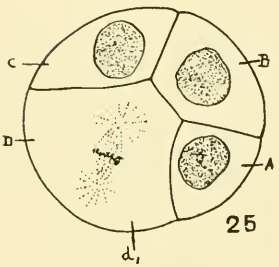

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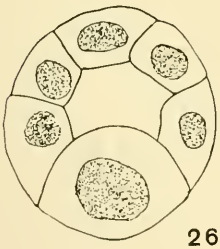
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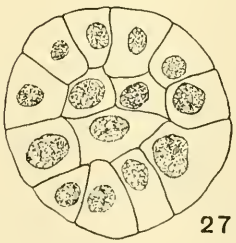
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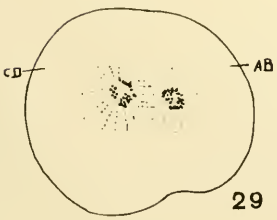

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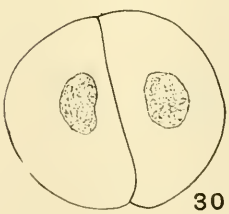
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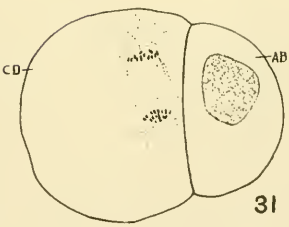


31

PLATE 4

EXPLANATION OF FIGURES

All the figures on this plate are from parthenogenetic eggs.

32 and 33 Three-cell stage.

34 Four-cell stage.

35 Five-cell stage.

36 Nine hours after treatment.

37 Twenty-four hours after treatment.

38 to 41 Multinucleate stages.

42 One polar body. Telophase first cleavage.

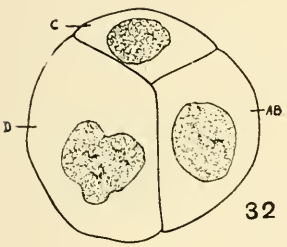
43 One polar body. Two-cell stage.

44 to 47 Abnormal polar divisions.

47 Three polar bodies. Two-cell stage.

48 Two polar bodies. Four-cell stage.

Magnification, 575 diameters.



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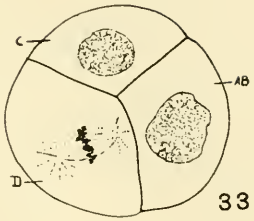

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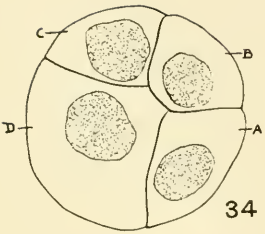

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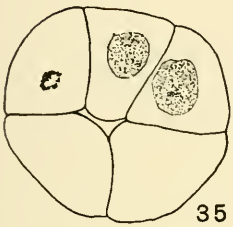
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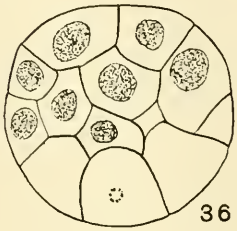
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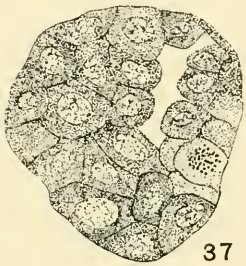
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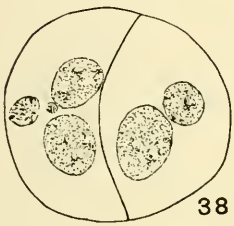
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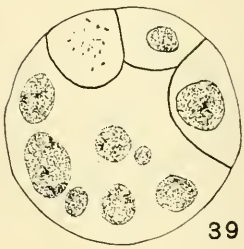
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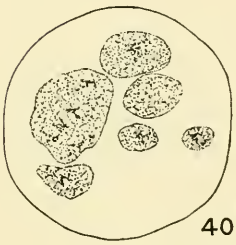
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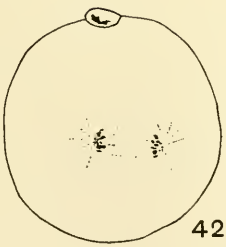
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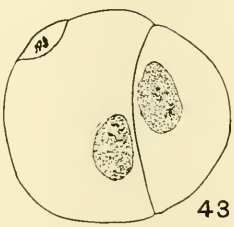
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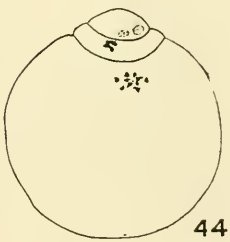
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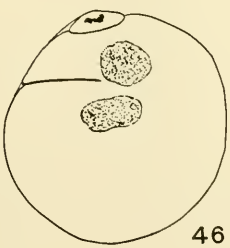
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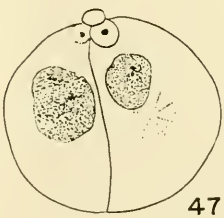
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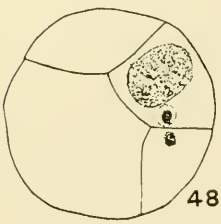
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PLATE 5

EXPLANATION OF FIGURES

All the figures on this plate are from parthenogenetic eggs.

49 Metaphase, first polar spindle.

50 Equatorial plate, first polar spindle.

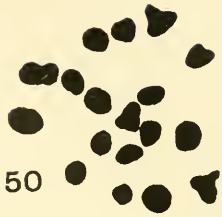
51 to 53 Anaphase, first polar spindle.

54 to 56 Telophase, first polar spindle.

57 Resting nuclei from the first polar spindle.

Magnification, 2600 diameters.

49



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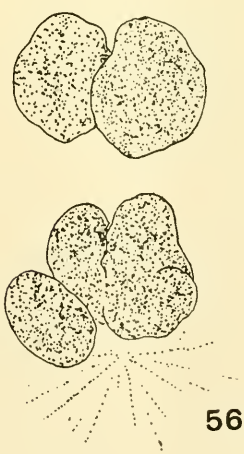

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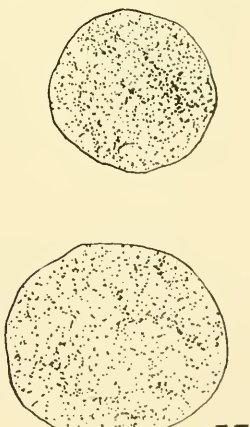

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PLATE 6

EXPLANATION OF FIGURES

All the figures on this plate are from parthenogenetic eggs.

58 Resting nuclei enlarging.

59 Resting nuclei fusing.

60 Cleavage nucleus.

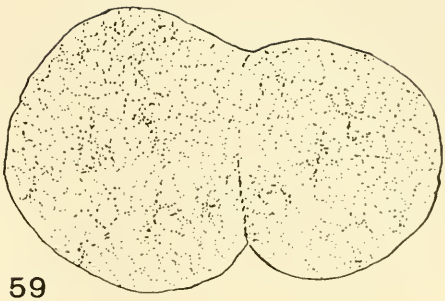
61 Cleavage nucleus. Chromatin condensing.

62 Cleavage nucleus. Chromatin in threads.

63 to 65 Chromosomes of the first cleavage from eggs without polar bodies.

Magnification, 2600 diameters.

58



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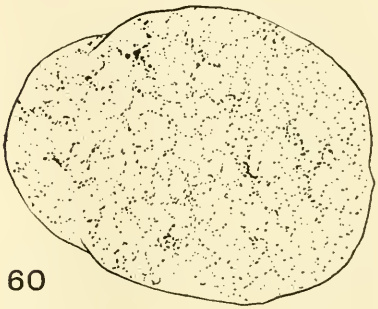

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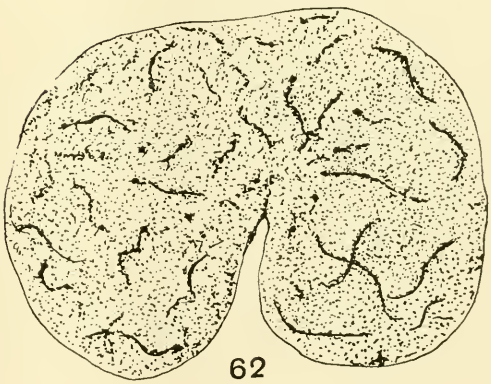

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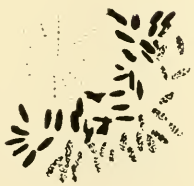

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PLATE 7

EXPLANATION OF FIGURES

All the figures on this plate are from parthenogenetic eggs.

66 Anaphase of first cleavage from an egg without polar bodies.

67 Chromosomes of second cleavage from an egg without polar bodies.

68 Chromosomes of middle cleavage from an egg without polar bodies.

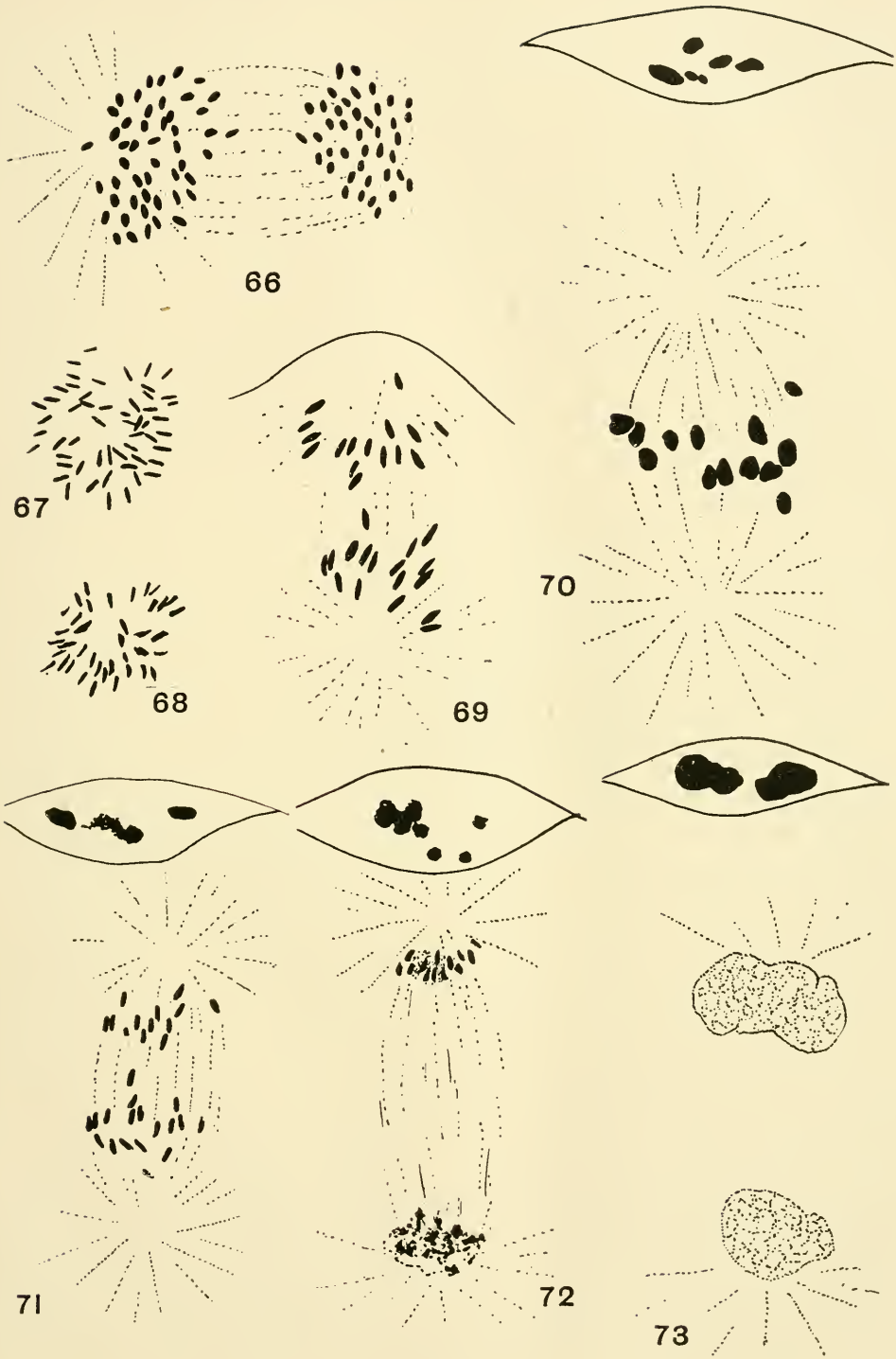
69 Formation of first polar body.

70 Metaphase, second polar spindle.

71 Anaphase, second polar spindle.

72 and 73 Telophase, second polar spindle.

Magnification, 2600 diameters.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70
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72
73

PLATE 8

EXPLANATION OF FIGURES

All the figures in this plate are from parthenogenetic eggs.

74 Resting nuclei from second polar spindle.

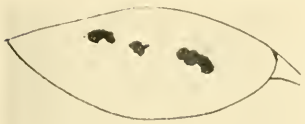
75 Cleavage nucleus of an egg with one polar body.

76 Vesicles in egg with two polar bodies.

77 Cleavage nucleus of an egg with three polar bodies.

78 Cleavage chromosomes, egg with two polar bodies.

Magnification, 2600 diameters.



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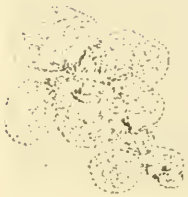

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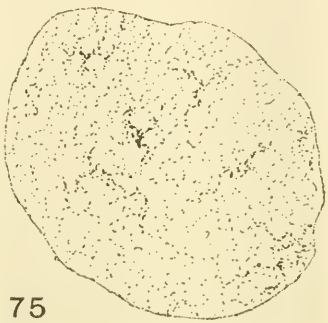

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78

ON THE REACTIONS OF AMEBA TO ISOLATED AND COMPOUND PROTEINS

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From the Zoological Laboratory of the University of Tennessee

SIX PLATES

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INTRODUCTION

Amebas have been shown to react positively to carmine, uric acid, india ink and old solid egg white (Schaeffer, '16, Jour. Exp. Zool., vol. 20, On the feeding habits of ameba). All these substances are comparatively easily soluble. Particles of these substances are not only eaten when the amebas come into contact with them, but all these substances are sensed at a distance of 100 microns or more and the usual result of such sensing at a distance is movement toward them. It may be presumed therefore that since these substances are soluble, the reaction at a distance to the solid particle is explained by the diffusion of the dissolved substance.

Now all these substances except uric acid are compound substances or mixtures of substances, and nothing is known of the rate of solubility of the various ingredients in them as they exist in the mixtures; it is therefore impossible to say just what part of the india ink or the egg white or the very attractive carmine affects the sense organs of the ameba or in what manner. Such reactions can shed but little light upon the problem of the selection of food.

It seems probable therefore that the use of isolated chemical compounds is to be preferred to the use of mixtures of substances in experimenting upon the feeding reactions of ameba because in this way the number of unknown factors is reduced to the minimum as far as the test substances are concerned. For this reason a considerable number of experiments were performed using as food substances globulin (crystallin and ovoglobulin) lactalbumin, ovalbumin, zein, keratin, and also a few compound proteins such as gluten, aleuronat and fibrin. From the chemist's practical point of view the isolated proteins mentioned can be made quite pure and quite insoluble. Three of the compounds, lactalbumin, ovalbumin, zein, were made under the direction of a chemist with long experience in this line of work and these products were made as pure as they can be made at present. The other substances were bought from dealers in chemical supplies.

But the insolubility of these as of all substances is doubtless relative, not absolute. The chemist, using his utmost skill, may not be able to detect a minute fraction of a quantity of substance going into solution, just as he is unable to detect odoriferous substance in a hare's foot-tracks. But these small quantities of material are frequently of the first importance to the student of sense perception. So while we shall apply the term 'insoluble' to globulin, lactalbumin, zein, ovalbumin, keratin, it should be remembered that not only the word but the meaning also is borrowed from the chemist. Speaking generally, we may not expect to be able to understand the relation between a sense organ and the substances which stimulate it by experimenting with two or three substances which are said to be pure. It is

more than probable that a large number of tests with a considerable number of substances will be necessary to detect and eliminate errors arising from incomplete knowledge of the physiological action of the test substances due to the presence of chemically imperceptible impurities.

Moreover the insolubility of some of the substances such as globulin, lactalbumin, ovalbumin, etc., is influenced to some extent by substances, organic and inorganic, held in solution in the water in which the amebas live. Since it is experimentally impossible to keep pure the water in which the amebas may be placed for observation, on account of their excretory products diffusing out, the proteins themselves may possibly become slightly soluble when these excretory substances come into contact with them. On the other hand it should be pointed out that the age of some protein compounds, especially albumins, affects their solubility—the older they become, the more insoluble.

In view of all these disturbing factors attending the use of proteins as test substances, it is evident that the observed reactions must be interpreted with care.

EXPERIMENTS WITH GLOBULIN

Merck's globulin with subtitle 'crystallin' was used. Whenever the word globulin is used unqualified in this paper this product is meant. A few trials were also made with a purer ovoglobulin on raptorial[1] amebas (the only kind of amebas obtainable at the time) but no definite results were procured. The solubility of Merck's globulin was tested by soaking for some hours a few small particles in a drop of distilled water on a clean glass slide and then evaporating the water. By examination with the microscope no difference could be observed between the slight amount of residue of such a drop of water and one in which no globulin had been soaked. Similar experiments were also made with tap water with similar results. If any of the globulin went into solution therefore, the amount was excessively small.

[1] See Postscript on p. 79.

Globulin was used in this work as the 'standard' food substance. This accounts for the frequent use of it in many of the experiments. The strength of the stimulus proceeding from any other substance may thus be compared directly with that of globulin. The degree of hunger or satiety may also be determined within certain limits in this way. That globulin is a real food substance undergoing solution (digestion?) inside the body of the ameba is shown in figures 532 and 534. The amebas were fed in filtered culture solution and occasionally in tap water.

Granular amebas

An ameba ate a piece of grain gluten (326–336,) then moved past another piece of grain gluten, then moved past this piece of grain gluten a second time. A piece of globulin was then laid in its path (366). The ameba flowed directly into contact with the globulin, formed a food cup over it and ingested it. A second piece of globulin was partly surrounded as if ingestion was about to follow, but the ameba presently withdrew (377). Three and one-half minutes later, when the same grain of globulin was presented, it was promptly eaten (383). In this experiment globulin seems to have been somewhat more attractive than grain gluten.

A grain of globulin was placed near another ameba that had come to rest. It was moving only very slightly (387). At once two pseudopods were sent out toward the globulin, but they were withdrawn before they came into contact with it, the protoplasmic stream then turning to the left. The ameba continued flowing in this direction until the posterior part was being dragged past the globulin (399). The protoplasmic current was then reversed, and also a side pseudopod was thrown out on the right (now the left), which turned strongly toward the globulin until it came into contact with it. The side pseudopod was then withdrawn, and a little later another was formed on the left and anterior to the globulin. It also started to turn toward the globulin, but was at this stage retracted, whereupon the ameba moved past the globulin without further reaction.

This experiment is of interest in that it shows a gradual increase in intensity of hunger (the quiet condition of the ameba suggests the absence of hunger) until the side pseudopod came into contact with the globulin; then a decrease in intensity of hunger, leaving the ameba with even less intense hunger than at the beginning of the experiment. A somewhat similar result is observed in the following experiment, but infortunately only the first part of the process, the increase in the intensity of hunger, is recorded.

In the experiment just alluded to (393) the ameba moved gradually forward until the left limb (399) came almost into contact with the globulin grain placed before the ameba, when it was retracted and the ameba moved away. On the second trial with the same piece of globulin (402) the ameba again broke up into Y-form, the left pseudopod being directed toward the test substance (404). When the left prong came into contact with the globulin the right was retracted. The ameba then moved on without further reaction. Note that in the first trial with this ameba, the prong directed toward the globulin was retracted while the other prong became the main pseudopod; while in the second trial the reactions of the respective pseudopods were reversed. This difference in behavior shows that the intensity of hunger increased during the course of the experiment because of the presence of the globulin.

Similar to these two experiments is that recorded in figures 1616 to 1626. The ameba moved nearly into contact with the globulin (1619), then withdrew a short distance and threw out a pseudopod on the right. From this side pseudopod another was sent out which moved into contact with the globulin (1624). But as soon as the pseudopod came into contact with the test object, it was withdrawn and the ameba started to move away to the right; but streaming was soon interrupted and directed into the posterior end where a small pseudopod had been slowly forming. The posterior end now became the anterior. There can be little question that the ameba was not hungry and that the presence of the globulin heightened in this case the effect of surfeit. The experiment is of interest because of the striking

changes in protoplasmic streaming in the various pseudopods, which were provoked by the globulin. Action was not unified in 1624; the tendencies to move toward and away from the globulin were equally strong. But after a few seconds of contact with the globulin, the tendency to move away rapidly became the stronger. At this same time a small pod was forming at the posterior end, and it was doubtless this activity which finally prevented the ameba from flowing away through the right pseudopod, which appeared to become the main one. But the protoplasm of the posterior half would have had to flow toward the globulin to get into the pseudopod on the right. The necessity of this may have caused the ameba to flow away through the posterior end rather than through the pseudopod on the right.

The side pseudopods in figure 1623 illustrate graphically an internal condition affecting behavior that is frequently observed in amebas reacting indifferently toward food substances. When an ameba that is not hungry encounters a food particle which stimulates the ameba at the side of a pseudopod so that a new pseudopod is formed toward the food object, there is formed simultaneously or nearly so on the opposite side of the main pseudopod a pseudopod of about the same size and flowing at the same angle and at about the same rate as the one directed toward the food. Opposite pseudopods of this character are not formed by hungry amebas under otherwise similar conditions, nor by any amebas toward indifferent objects (glass, sand, etc.) nor toward objects producing usually negative behavior. There are in these reactions two tendencies present, a positive and a negative, judging from the objective behavior. The formation of pseudopods is not directly determined by the stimulating object as can be readily observed from the record of every experiment. Then how is pseudopod formation controlled? This question is of the profoundest interest.

An unusual method of ingestion in which two pseudopods were involved is shown in figures 939 to 950. The ameba was in Y-form with the globulin lying nearer the right limb. Nevertheless, the left limb enlarged the more rapidly and came first into contact

with the globulin. But when both prongs came into contact with the globulin (944) they started to surround it. The ameba was at this time in the form of a ring (945). The tips of the prongs fused as the globulin was ingested. The globulin was ingested in a food cup formed chiefly by the left prong. After ingestion the ameba rested for about twenty minutes, then moved off. Several similar instances have been observed.

Reactions to large pieces of globulin

In the experiments recorded above fragments of globulin small enough to be easily eaten were always employed. Let us now consider some experiments in which the globulin grains in every case were too large to be eaten.

In the first trial (408) the globulin grain was definitely avoided. The globulin was then shifted (412). As the ameba moved directly toward it, the antero-posterior diameter foreshortened, while the anterior edge broadened out considerably. Before the ameba came quite into contact with the globulin, a huge food cup large enough to enclose the test object was started (416). The ameba moved forward into contact with, and laid the partly formed food cup over the grain of globulin, to which the ameba then adhered with but slight movement for several minutes. After this period of comparative quiet, the ameba became more active in its movements and less of its body was in contact with the globulin. Pseudopods were then sent out successively in various directions (422 to 426) only to be retracted later. Finally one was sent out (429) which led the ameba away from the globulin, but only after the globulin had been dragged for a considerable distance. The ameba was in contact with the globulin for an hour and nine minutes. One minute later the globulin was shifted (433). The ameba flowed directly into contact with it, and remained so for eleven minutes. There was no attempt at the formation of a food cup, nor was the globulin adhered to with avidity. On the fourth trial the ameba avoided the globulin (445). On the fifth trial (450) the ameba moved into contact with the globulin and re-

mained in very loose contact, without at any time quieting down, for fourteen minutes. On the sixth trial the ameba moved into contact with the globulin at its side (457). The ameba remained in rather loose contact with the globulin for about twelve minutes. The same piece of globulin was, for the seventh time, placed ahead of the ameba (467). As the ameba moved forward it broke up into several pseudopods, indicating negative behavior; but presently one of them moved forward some distance, then turned to the left and toward the globulin, and finally moved into contact with it. The ameba remained in loose contact with the globulin for about six minutes.

A half minute later a new piece of globulin was laid in the ameba's path (482). At first the ameba reacted decidedly negatively and moved on past the globulin. But a pseudopod was then sent out on the right which was directed toward the globulin and which carried the ameba into contact with it. The globulin attracted the ameba strongly but not so strongly as did the other piece in the first trial. The ameba remained in contact with the globulin for about thirty-seven minutes. For the sake of comparison a small piece of fresh globulin was laid near the ameba, now in Y-form (496). The globulin was ingested promptly without the formation of a food cup.

Twenty-three hours later the ameba was found to be in normal condition. A large piece of fresh globulin was laid in its path (502). The ameba reacted negatively at first (503) but it happened that as the ameba toppled over (it had been standing on end) it came near the globulin to which it was then strongly attracted. The ameba had been in contact with the globulin for an hour and twenty-two minutes when observations were terminated. A number of times the ameba had almost broken away, but each time it returned again to the globulin.

These observations on the behavior toward large food masses show clearly: (1) that the size of the food object plays little part in selection; (2) that globulin has the power of initiating the formation of a food cup before the ameba has come into contact with it; (3) that the globulin particle becomes changed by being

in contact with the ameba, for the same ameba remained in contact with the same grain of globulin for much shorter periods after the first test; but when a new grain of globulin was introduced the period of contact again became much longer; (4) that if the first or any trial produces negative behavior leading to avoidance of the globulin, the following trial is quite likely to show positive behavior; (5) that three hours of almost uninterrupted contact with a large piece of globulin has practically no effect on efficient feeding (496) but it is possible that the feeding reaction is a little less intense, that is, instead of a food cup being formed, the globulin is merely surrounded by protoplasm without including water. The formation of a number of small pseudopods at the anterior end of the ameba in figures 498 and 499 also indicates a mildly disagreeable effect due to the ingestion of the globulin.

Raptorial amebas

Raptorial amebas are not attracted by globulin as strongly as the granular amebas are; it is only occasionally that a grain of globulin is eaten at all, and if eaten, is soon excreted.

In the path of an ameba from a wild culture, which had been tested with crystallized egg albumin, was placed a grain of globulin (2293). The ameba moved forward toward the globulin for a short distance, then stopped abruptly and broke up into several pseudopods which are numbered from 1 on in the order of their formation, to facilitate description. Pseudopod 1 moved toward the globulin a short distance, then formed 6; but both were then immediately withdrawn as 3 rapidly enlarged (2296, 2297). No. 7 was then projected. It curved backwards slightly and attained to considerable size. It was presently arrested in its development as 6 became active again and moved rapidly toward the globulin grain and ingested it in a typical, though probably incomplete, food cup. Pseudopods 1, 2 and 5 had completely disappeared, but the vestiges of 3, 4 and 7 were very conspicuous (2301). Without a period of rest or quiet after the formation of the food cup, 7 suddenly became active

and enlarged to a considerable extent, but it was arrested as the inconspicuous vestige of 4 suddenly became activated (2302, 2303). The ameba finally moved away through this pseudopod. The globulin was left behind seven minutes after the food cup was begun.

This experiment is an illustration of that comparatively rare occurrence under experimental conditions where the tendencies toward positive and negative behavior, with regard to a definite object, are nearly evenly balanced. We see here a change from negative (2295) to positive (2298 to 2301) to negative behavior again (2302 to 2304). Of so many changes there can be no doubt. But it is possible that there were more fluctuations between negative and positive reacting. Thus figures 2293 and 2296 may also represent positive states. But even if these latter fluctuations are left out of account, there is offered here a remarkable opportunity of gaining an insight into the nature of ameban behavior which is not possible where reaction is decidedly and uniformly either negative or positive.

In figure 2294 is seen the first indication of a negative reaction toward the globulin, viz., the interruption of concerted streaming and the slight turning to the right. The formation of a number of pseudopods in 2295 indicates a still stronger negative reaction which reaches its maximum in 2297. The behavior was thus far not unified, for the slight forward movement of pseudopods 1 and 6 in figure 2296 may be regarded as evidence of positive behavior. With the retraction of these pseudopods and the enlargement of 3, the behavior may be said to be negative, involving the whole ameba. The formation of 7 shows a change to positive behavior again and the ameba as a whole began to react positively in a concerted manner, as is indicated by the fact that the vestige of 6 became active again leading the ameba directly toward the globulin. No. 4 also increased in size slightly. All the others, including 4, were slowly retracted when the globulin was reached. The resumption of streaming in 7 indicates a reception of disagreeable stimuli from the globulin (2302). The retraction of 7 and the rapid enlargement of 4 indicates more intensely disagreeable stimuli proceeding from the globulin

(2303). This again leads to a maximum condition of negative behavior. Why was pseudopod 7 withdrawn and 4 reactivated in figure 2302? It looks like an unnecessary change in behavior. Why should not the ameba have flowed away from the globulin through 7? What were the factors which determined that 4 should be the one through which to move away? Or why was not a new pseudopod formed through which to move off? The reaction involved in this case is possibly adaptive. No. 4 was a nearer 'exit' for the strongly negatively stimulated protoplasm in 6, than was 7. The only exit nearer than 7 was 4; any other exit would have necessitated the formation of a new pseudopod, which does not happen when the young vestige of a previous pseudopod in the vicinity can be reactivated. Flowing away through 7 (2302) was making of 6 the posterior end of the ameba, a region of comparative inactivity in streaming; while flowing away through 4 restored the posterior end in its original place, and made possible thereby a more rapid flow of protoplasm from 6 and also restored the original direction of movement (South) of the ameba. This suggested explanation involves no elements of behavior which can not be shown to exist in the reactions of ameba. Flowing away through 4 instead of through 7 afforded the quickest relief from a disagreeable situation.

Summary of reactions toward globulin

Amebas sense globulin grains at a distance of at least 100 microns. If the globulin grain is a small one the reaction is nearly always positive, whether the globulin is subsequently eaten or not. If the globulin grain is large the reaction may be negative when first sensed at a distance. Both large and small grains call forth the feeding reaction. Usually the feeding process is initiated only after the ameba has come into actual contact with the globulin, but occasionally a food cup is started before the ameba comes into contact with the globulin.

Feeding is usually accomplished by means of food cups containing variable quantities of water. But in some cases the protoplasm merely flows around the globulin. Differences in the

intensity of hunger determine, at least to some extent, whether the globulin shall be eaten by means of a food cup or not, and whether the quantity of water taken in with the globulin shall be large or small. Intense hunger calls forth large food cups; mild hunger, small food cups with little water; and very slight hunger may prevent the formation of food cups altogether, the protoplasm merely wrapping itself around the globulin.

Globulin is an actual food substance. Grains of it undergo gradual but marked diminution in size in the body of the ameba. Globulin is disintegrated somewhat more slowly than fragments of aelosoma meat. On account of its food properties globulin has been used in these experiments as the 'standard' test substance by which the attractiveness of other substances may be compared and estimated. Of all the chemically prepared and isolated substances used in this work, globulin is the most attractive, excepting perhaps carmine. Whenever globulin is eaten and retained the inference is that the ameba is hungry.

The experiments in which large pieces of globulin were used show that if an ameba comes into full contact with a single piece several times in succession, the globulin becomes less and less attractive, just as carmine does if a piece is ingested repeatedly. Just what causes this change in behavior is not known. These experiments also show that particles much too large to be eaten call forth typical feeding reactions.

The granular and the raptorial amebas differ strikingly in their behavior toward globulin; the granular eat it with great readiness while the raptorial amebas seldom eat it, and in many cases after it is eaten egestion soon follows. This indicates a considerable and definite difference between these forms, but whether this difference is hereditary of acquired has not been determined.

Several of the experiments show quite clearly that in raptorial amebas disagreeable stimuli may proceed from globulin after it is ingested. No definite explanation can be given at present of these exceptional cases.

EXPERIMENTS WITH GLUTEN

This substance was purchased from Eimer and Amend. Its purity was not investigated. It is probable that several proteins are included under the term gluten. The particles of gluten, when immersed in water, showed a brownish tint under the microscope, while those of globulin were a light straw yellow. The results of the experiments with this substance are in most respects similar to those in which globulin was used.

The attractiveness of grain gluten as compared with globulin is well shown in the following experiment. A grain of gluten was placed in the path of a granular ameba (326). The ameba moved on into contact with the gluten and then on under it. Presently the gluten rolled off the ameba's back. The ameba then surrounded it without forming a food cup. The eating process suggested only mild attraction toward the gluten. Four minutes after ingesting the gluten the ameba moved off in the original direction. A small ameba of another species that happened to be near, attracted the ameba slightly, but it finally turned to the right and avoided the small ameba. Another grain of gluten that was now placed in the ameba's path was avoided at first but attracted the ameba later. It was nevertheless finally left behind as the ameba moved away. The gluten grain was placed in the ameba's path again but was passed by without ingesting it. The gluten was laid in the ameba's path for the third time but it was passed over with seeming indifference. Next a grain of globulin (366) was eaten. A fresh piece of globulin placed before it was also ingested. A third piece of globulin remained unnoticed (381). But when this piece of globulin was placed near the ameba it was promptly ingested (383).

Globulin evidently attracted this ameba considerably more strongly than gluten did, three pieces of globulin having been eaten to one of gluten. Moreover the gluten grain was not eaten with readiness but with indifference; while globulin was eaten as if the ameba was very hungry. In the five tests with gluten to which the ameba was subjected, behavior became more and more indifferent with each succeeding trial. It is likely that the habit of behaving indifferently, or negatively, to the gluten

caused the negative behavior when the first grain of globulin was presented (372). The negative behavior was certainly not due to lack of hunger, as the succeeding experiments show; and negative behavior was also absent at the beginning of this series of experiments, when gluten was eaten. The most reasonable explanation therefore is that repeated contact with a substance (gluten) possessing comparatively mild food qualities developed a negative condition which had first to be overcome by stimulation by the stronger food qualities of the globulin.

A raptorial ameba which had been treated with several other substances was tested with a grain of gluten (1765). The ameba moved toward the gluten and then passed it on the right without coming into contact with it. Two opposite side pseudopods indicating positive and negative tendencies of reaction (see p. 58) were then formed of which the left one moved into contact with the gluten and rolled it along for some distance (1769, 1770). The large pseudopod which was formed on the convex and right side of the left member of the two opposite pseudopods (1767, 1768) indicates nearly a balance between positive and negative tendencies of reaction. Had the gluten grain not rolled away as the left pseudopod moved against it, the ameba would probably have moved off through the large pseudopod formed on the right side of the left pseudopod. A pseudopod was thrown out on the right through which the ameba started to move away (1772) without having shown any inclination so far to eat the gluten; but on agitating the gluten slightly with a glass needle, a food cup was partly formed over it (1773). Ingestion however did not follow. The ameba retracted the food cup and moved on.

Another raptorial ameba with a long experimental record in which a considerable number of tests were made with various substances and which had partly ingested a grain of aleuronat and a grain of carbon (unagitated) and wholly ingested a grain of globulin, was then presented with a grain of gluten (1872). The ameba flowed directly into contact with it, formed a typical food cup and ingested it (1878). Two pseudopods were sent out presently from the outer rim of the food cup with the gluten in the crotch that was thus formed (1879). The gluten was partly

exposed at this time, showing that it had been incompletely ingested. The pseudopod on the left was withdrawn as the gluten was incompletely reingested (1880). As the ameba moved on the gluten was finally brought up to the posterior end only slightly in contact with the protoplasm (1881, 1882). Protoplasmic streaming was now reversed and the gluten reingested (1883) but again incompletely, for as the ameba resumed movement in the original direction, the gluten was left behind without further reaction toward it (1884 to 1886). A grain of globulin was then placed in the ameba's path (1887). The ameba moved forward into contact with it, then reversed streaming in the main pseudopod, then broke up into several pseudopods and finally moved off to the left (1890). The globulin grain was then shifted (1891). A food cup was then formed and the globulin ingested in typical manner. The ameba moved off through a vestige of one of a pair of opposite pseudopods on the left indicating nearly a balance between negative and positive behavior (cf. especially with figures 2293 to 2304), without a period of rest, leaving the globulin behind (1893 to 1896). A piece of gluten was then laid in the path of the ameba but it was avoided. On the second trial with the gluten the ameba reacted toward it with indifference.

The behavior just described may be regarded as typical for a hungry raptorial ameba. These animals are stimulated at a distance by such substances as globulin, gluten, aleuronat, etc., so that they move toward these objects. In many cases the feeding reaction is initiated though it is seldom completed. It seems that movement of the object after it is enclosed, is essential to complete ingestion. In all the experiments performed upon this ameba only one test object was eaten: globulin (not reported here). It is almost certain that this would have been excreted soon thereafter but for the ingestion of a flagellate immediately afterward, for no period of rest ensued after ingestion and the direction of movement was changed after ingestion. I have frequently observed that the eating of a flagellate or other living organism has such an effect.

The causes determining the behavior in figures 1878 to 1886 are not entirely clear. It is possible that when the gluten was partly exposed (1879 to 1881) the motion of the ameba caused the free surface of the gluten to come into contact with other parts of the ameba, giving thus the effect of movement of the gluten and producing therefore an efficient food stimulus. For this ameba, gluten lost its stimulating food properties as soon as it was surrounded by a food cup; but when the food cup was withdrawn and the gluten was again exposed, it recovered its stimulating qualities. Although a motionless object may be ingested, it will not remain ingested as a rule unless either the object for some cause moves about for a short while or another moving subject is eaten soon thereafter.

Summary of behavior toward grain gluten

In general, the grain gluten used in the foregoing experiments calls forth reactions that are very similar to those produced by globulin, excepting that globulin is somewhat more attractive than gluten.

Gluten is an actual food substance which undergoes digestion in the ameba's body considerably more rapidly than globulin, as is indicated in figures 532 and 534.

Granular amebas eat gluten more readily than the raptorial; the latter seldom completely ingest solid dead bodies of any sort, and gluten forms no exception. As a rule the eating process goes on normally until the gluten is almost surrounded with protoplasm. Streaming then begins in some other part of the ameba, usually in the vestige of a previous pseudopod, and the ameba then moves off leaving the gluten behind. This break in the ingesting process seems to be due to the lack of stimulation such as is produced by movement of the food object. Apparently it requires stronger (or different) stimuli to bring about this last stage in feeding than any other, for if the object is once completely surrounded by protoplasm and the edges of the protoplasmic covering sheet have fused, it is almost invariably retained and digested, if of food value. That the lack of requisite stimulation to complete ingestion proceeds from lack of move-

ment was pointed out when the ingestion of living organisms was discussed in a previous paper (On the feeding habits of ameba, Jour. Exp. Zoöl., vol. 20, 1916). This point will be discussed again from another point of view in a later paper.

Although movement seems to be necessary in an object in order to insure its being eaten, yet movement is unnecessary to attract a raptorial ameba from a distance; and in exceptional cases movement seems to be unnecessary even to successful eating. The changes in ameba which give rise to these cases of exceptional behavior are not understood, owing to the small number of recorded experiments.

EXPERIMENTS WITH ALEURONAT

This is a commercial product and consists of several substances in intimate mixture. It is sometimes used in the dressing of wounds on account of its so-called chemotactic action on leucocytes. The greater part of aleuronat consists of proteins. The food qualities of aleuronat are similar to those of the other insoluble proteins mentioned above, as the following experiment will indicate.

In the path of a granular ameba was placed a grain of aleuronat (1372). The ameba moved directly forward and as it neared the aleuronat it spread out. Two pseudopods were then sent out at the edges of the main pseudopod, which finally enclosed the test substance in an imperfect food cup. The aleuronat was completely ingested. One and one-half minutes later the ameba resumed its previous direction of movement. The ameba reacted toward aleuronat as it does toward globulin or any other lifeless food body. But the stimulating power of alueronat does not seem to be intense, for no food cup was formed. As compared with a piece of egg white, fed ten and one-half minutes later, which was ingested with a large food cup, aleuronat seems to possess only mild food properties.

Granular amebas eat aleuronat but not with the same readiness with which gluten and globulin are eaten. In other respects it calls forth about the same behavior as these substances. Raptorial amebas do not eat aleuronat. In some cases ingestion is

begun, but not completed. The food stimuli proceeding from aleuronat do not seem to affect amebas as intensely as those arising from gluten or globulin. All amebas sense aleuronat at a distance and usually move toward it and frequently into contact with it. In consequence the direction of movement is often changed. The digestibility of aleuronat has not been tested.

EXPERIMENTS WITH LACTALBUMIN

The lactalbumin used in these tests was coagulated in the process of preparation and may therefore be regarded as insoluble in water.

Sixteen experiments with lactalbumin as the test substance were performed upon a granular ameba. Previous to these experiments the ameba ate a piece of globulin and also a grain of keratin, but the keratin was excreted a few minutes after its ingestion. Following several other experiments with keratin, there was placed in the ameba's path a grain of lactalbumin (695). The ameba avoided it and moved on. The lactalbumin was then shifted so as to lie again in the ameba's path (699). The ameba moved directly forward, showing that it was attracted somewhat, or at least not repelled, by the lactalbumin. The ameba came into contact with the test substance at the side and partly passed by it, but two side pseudopods were then sent out against the lactalbumin. These formed a large food cup in which the lactalbumin was slowly ingested. The ameba then became comparatively quiet for about two minutes. Five minutes after the formation of the food cup the lactalbumin was egested, the ameba moving off in the original direction (707). A few minutes later a new piece of lactalbumin was laid in the ameba's path (708). The ameba moved forward passing the lactalbumin on the right for a short distance. A typical food cup was then formed in a manner not quite like the first, in which the lactalbumin was ingested (713). The ameba remained comparatively quiet for a few minutes. About seven minutes after the formation of the food cup the lactalbumin was excreted (718). A fresh piece of lactalbumin was then placed ahead of the ameba (719). The ameba moved directly into contact with

it and then started to pass it on the left. An imperfect food cup was then formed in a rather peculiar manner, in which the lactalbumin was ingested (723, 724). The ameba did not quiet down after ingesting the lactalbumin but moved on as if ingestion had been a side issue. A little later (726) the ameba flowed on through a new pseudopod formed on the right. The lactalbumin was excreted about three minutes after it was eaten. A new piece of lactalbumin was then laid in the ameba's path (730). The ameba moved forward and passed on the right of it. The numerous small pseudopods at the anterior end (731) indicate nearly a balance between negative and positive tendencies of reaction. When the tip of the ameba reached the further side of the lactalbumin, it swung to the left as if to encircle the lactalbumin. A food cup was then formed in which the test object was eaten. The ameba did not become quiet but after throwing out and retracting several pseudopods (735 to 737) moved off 135° to the left and excreted the lactalbumin about four minutes after ingesting it. The same piece of lactalbumin was again laid in the ameba's path (740). After the ameba had moved into contact with it, the direction of movement was changed slightly and the ameba moved on avoiding the test object. A new piece of lactalbumin was then laid in the path of the ameba (744). The ameba moved forward and started to pass it on the right, but a pseudopod was then sent out on the left and posterior to the lactalbumin, which was then ingested with very little water and without the formation of a definite food cup. The ameba moved off without a period of rest and excreted the lactalbumin about two and one-half minutes after ingesting it. The same piece of lactalbumin was again laid near the ameba (754). The ameba sent out a pseudopod into contact with it and kept on following it as it rolled away while being pushed around by the ameba. But the lactalbumin was laid for the third time in the ameba's path (764). The ameba moved toward the test object and then passed it on the right. A side pseudopod was sent out anterior to the lactalbumin which was then followed around for a short while as it rolled away from the advancing pseudopod. The ameba finally moved off through a newly formed pseudopod,

leaving the lactalbumin behind. Once more the same piece of lactalbumin was laid in the ameba's path (774). The ameba moved forward and passed it on the left. A side pseudopod was thrown out on the right just anterior to the lactalbumin, when the lactalbumin lay near the mid-region of the ameba. The lactalbumin was partly surrounded by protoplasm when it came near the posterior region, but it was released as the ameba moved forward. In the next experiment a new piece of lactalbumin was brought near the tip of the ameba (780). The ameba moved straight forward into contact with the lactalbumin and then started to pass it on the right. But when the tip of the main pseudopod had moved beyond the test object, a pseudopod was thrown out toward it. The lactalbumin was ingested apparently, but the ameba did not quiet down nor change its direction of movement. One and one-half minutes later the lactalbumin was left behind. The same piece of lactalbumin was again laid near the anterior edge of the ameba (789). The ameba passed it on the right. A pseudopod was sent out anterior to it when it came to lie in contact with the mid-region of the ameba. The test object seemed again to be partly surrounded by protoplasm when it came to lie near the posterior end, precisely as was observed in figures 774 to 777 in similar circumstances. Finally the ameba left the lactalbumin behind. But the test object was again laid in the ameba's path (795). The resulting reactions were very similar to those in the experiment just preceding. The lactalbumin was then for the fourth time laid in front of the ameba (800). The behavior that was observed was again very similar to that of the two preceding experiments. To test the degree of hunger a piece of globuln was next presented (806). Although it was eaten at once in a typical food cup, no resting period followed. A fresh grain of lactalbumin was then laid near the tip of the ameba (813). The ameba flowed past it on the right. A pseudopod was sent out anterior to it when it had come to lie in contact with the mid-region of the ameba. When it came to lie at the posterior end of the ameba it was surrounded by protoplasm and ingested. The ameba then became a little less active for a few minutes, but it finally moved away in the

same dirction leaving the lactalbumin behind three and one-half minutes after ingestion. The same piece of lactalbumin was then presented again (823). The ameba moved forward into contact with it, then followed it about for a while and finally left it behind.

The general behavior of this ameba is striking in several ways. Globulin is readily ingested to remain so, but lactalbumin is invariably excreted after ingestion. Likewise keratin. After a lactalbumin grain has been once ingested it is not ingested again, although it may attract the ameba into following it about for a while. If the ingested and excreted grain of lactalbumin is presented several times in succession, each succeeding test calls forth less change in behavior; but if followed by a fresh grain ingestion may ensue. The decreasing attractiveness of the same lactalbumin grain when presented several times in succession, may be due to learning of a simple sort. This ameba is fairly consistent in its general behavior. Figures 744 to 779 present behavior very similar to that recorded in figures 780 to 805.

Another ameba from the same culture reacted to lactalbumin in a different manner; several grains of it were completely ingested to remain in the body for a considerable time. The first grain was ingested in a typical food cup (854). Ingestion was not followed by a period of rest. After several pseudopods had been thrown out and retracted, the ameba finally moved off in the original direction. A fresh piece of lactalbumin was then laid in the ameba's path (87). The ameba came into contact with it at the side. The lactalbumin was ingested by the protoplasm flowing around it. There was again no period of rest after eating the lactalbumin. The ameba moved off 30° to the right, carrying the two pieces of lactalbumin at the extreme posterior end of the body. A third piece of lactalbumin was then laid in the path of the ameba (883). The ameba flowed directly toward it and ingested it in an imperfect food cup. The ameba moved off without a period of rest 60° to the left of its original direction. This left the last grain of lactalbumin at once in the posteroir end of the ameba. But the lactalbumin was not subsequently excreted. A grain of globulin was then presented for purposes of comparison (888). It was ingested in an imperfect

food cup. The ameba became much less active after ingestion of the globulin. Movement was slow and somewhat irregular. Another piece of lactalbumin was then laid near the ameba (896). The ameba moved into contact with it and then the behavior became very irregular. The lactalbumin was once partly surrounded, but was finally left behind as the ameba moved on. A few minutes later another piece of lactalbumin was placed in the path of the ameba (904). The ameba moved directly forward into contact with it and formed a typical food cup in which it was ingested. Without quieting down, a pseudopod was thrown out on the right, which elongated to a considerable extent. The posterior end then became active. Finally after some minutes the ameba, in spatulate form, moved away in the original direction.

Summary of reactions to lactalbumin

The experiments described above were performed on two granular amebas, both coming from the same culture. Both of these amebas readily ingested lactalbumin, but they differed in the length of time the ingested lactalbumin grains were retained. Both amebas retained globulin. The first ameba retained no lactalbumin, the second all that was eaten. But the contrast in this respect between the two amebas is not as significant as might appear. The second ameba seemed disturbed after the ingestion of the last two grains of lactalbumin. The original direction of locomotion was changed so that the lactalbumin came to lie at once in the posterior part of the ameba. This is particularly noticeable in figures 908 to 911. It may be concluded therefore that the tendency to get rid of the lactalbumin was present in the second ameba, although it was not sufficiently strong to cause excretion. It should be pointed out that the first ameba seemed to become disturbed by the ingested globulin (808 to 812) and that the second ameba did not eat the globulin with great readiness. It appears then that the stimuli inducing feeding are considerably less intense in lactalbumin than in globulin, and that after ingestion these stimuli seem to have but slight effect on the endoplasm and in some cases no effect.

The digestibility of lactalbumin has not been tested.

EXPERIMENTS WITH ZEIN

By reason of the fact that this protein is soluble in 95 per cent alcohol, it may be obtained in a state of great purity. Zein is insoluble in water. This is one of the purest proteins obtainable.

In the path of a granular ameba which had previously eaten two grains of globulin but which was unable to retain lactalbumin, although several pieces had been ingested, was placed a grain of zein (833). While the ameba was moving forward toward the zein, a pseudopod was formed on the right through which the ameba moved off. A fresh grain of zein was then placed ahead of the ameba (836). The ameba moved forward into contact with it and then pushed it about for awhile. A third grain of zein was then placed in the ameba's path (843). The ameba flowed into contact with it and then rolled it about for a while. At one time it seemed as if ingestion were about to occur (850, 851); the zein was partly surrounded but the protoplasm was soon withdrawn from it, and the ameba moved on, leaving the zein behind.

In the path of a granular ameba from the same culture as the preceding, after reacting as recorded in figures 854 to 911, was laid a grain of zein. The ameba moved off in another direction, no attempt being made to ingest it.

Zein does not seem to be sensed at a greater distance than sixty microns. The short range at which this substance may be sensed is possibly due to its freedom from soluble impurities. In none of the experiments was there any certain attempt at ingestion, although in several cases the zein was rolled around for awhile. As a stimulator of the feeding mechanism zein stands far below lactalbumin. As will be seen later, zein really stands at the bottom of the list of all isolated proteins in this respect. It seems to have no more power to induce feeding than particles of sand.

EXPERIMENTS WITH OVALBUMIN

The ovalbumin was coagulated in the process of preparation to render it insoluble.

In the path of a granular ameba that had previously ingested a piece of globulin was laid a piece of ovalbumin (not figured).

The ameba moved directly forward toward it a short distance, then turned to the left. Several pseudopods were sent out on the right but all of them were soon withdrawn. Finally the tip of the ameba turned strongly to the right and through it the ameba moved away. The same grain of ovalbumin was again placed in the path of the ameba (2072). The ameba moved partly past it on the right. A food cup was then formed and the ovalbumin ingested. About six minutes after the formation of the food cup the ovalbumin was left behind.

A fresh grain of ovalbumin was laid in the path of another granular ameba (2082). The ameba moved forward a short distance, then broke up into two pseudopods of which the one nearer the ovalbumin became the main pseudopod. As the ameba moved forward, a side pseudopod was thrown out on the right. This pseudopod formed the left limb carrying the ameba away. There was again a slight tendency to encircle the ovalbumin.

Ovalbumin seems to stand between lactalbumin and zein in its power to stimulate the feeding reaction. In contrast to zein, ovalbumin provokes ingestion frequently, but not so frequently as lactalbumin. Ovalbumin also is sensed at a greater distance than zein—about 100 microns. The digestibility of ovalbumin was not tested.

EXPERIMENTS WITH KERATIN

This albuminoid was of Merck's manufacture. When freed from fats and other proteins keratin is insoluble in water although it swells up slightly. A grain of keratin was placed directly ahead of a granular ameba (631). The ameba moved forward into contact with it and partly surrounded it, but the ameba kept on flowing in the original direction, and in a few minutes the keratin was left behind. A new piece of keratin was then laid near the ameba (640). The ameba moved forward toward the keratin and when nearly in contact with it, broke up into two pseudopods, both of which advanced toward the keratin until they came into contact with it. The left pseudopod then enlarged the more rapidly and moved on, leaving the keratin

behind. A grain of globulin was then promptly ingested. Another grain of keratin was then laid in the ameba's path (657). The ameba flowed into contact with it and ingested it by slowly flowing around it. The ameba became comparatively quiet for a few minutes. The keratin was then excreted but at once reingested (677). Within a minute and a half after reingestion it was again excreted and left behind. A new piece of keratin which the ameba encountered (679) as the previous piece was excreted, was avoided, although a small pseudopod was sent out toward it. Another new piece of keratin was placed in the ameba's path (682). The ameba moved toward it slowly, then encircled it almost completely and finally moved off. The ameba had not come into contact with the keratin. This is one of the best instances on record of the encircling of an object by an ameba without coming into contact with it. This phenomenon is of the greatest interest but discussion of it must be deferred until later.

A piece of keratin was placed in the path of a slow moving raptorial ameba (951). The ameba moved almost into contact with the keratin, then turned toward the left and moved on. The keratin was shifted but it was again avoided (957). The same piece of keratin was again placed before the ameba (960). The ameba moved on over the keratin without any definite reaction toward it. A fresh piece of keratin was next laid in the path of the ameba (965). The ameba moved into contact with the keratin and formed an imperfect food cup in which the keratin was ingested. Two hours later the keratin was still present in the ameba's body.

The general behavior of ameba toward keratin is very similar to that of ovalbumin. On account of the paucity of experiments no accurate comparisons may be made, but in a general way it may be said that keratin and ovalbumin seem to stimulate amebas with about equal intensity. The fact that a raptorial ameba retained a keratin grain for over two hours recalls similar results with carmine. Raptorial amebas are much more apt than the granular to retain what they ingest, whether the ingested substance is of food value or not. It may be of little significance therefore that keratin was retained for hours after ingestion by a

raptorial ameba. If it had been a granular ameba such behavior would have been significant, for it would not have been expected. Keratin can be sensed at a considerable distance, apparently about 100 microns or more.

EXPERIMENTS WITH FIBRIN

The fibrin used was of a commercial variety and probably not carefully purified. It is however a very insoluble substance, and for exploratory work its use may be permissible.

In the path of a raptorial ameba that had eaten a grain of globulin was placed a grain of fibrin (1004). The ameba moved toward the fibrin but before it came into contact with it a pseudopod was thrown out on the left through which the ameba moved away. The same grain of fibrin was eaten (imperfectly?) when agitated, but was excreted four minutes thereafter.

In another experiment, not illustrated in this paper, a grain of fibrin was eaten by a raptorial ameba in normal manner. Three minutes thereafter a flagellate was eaten. Eight minutes after this another flagellate was ingested at the posterior end where the fibrin lay. But in spite of these hindrances to the excretion of the fibrin, it was egested sixteen minutes after it was eaten.

Fibrin ranks with ovalbumin and keratin in the qualities that stimulate the feeding reactions.

SUMMARY

1. Ameba eats isolated proteins. Globulin (crystallin) is eaten quite readily and particles of it undergo reduction in size in the body. Lactalbumin is sometimes eaten, but it is not nearly as attractive a food as globulin. Ovalbumin is even less attractive than lactalbumin and is eaten only occasionally. Zein, which can be more readily freed from impurities than the other proteins mentioned, attracts amebas to move toward particles of it but in no case does ingestion follow. It is possible therefore that the attractive qualities of ovalbumin and lactalbumin are due to minute traces of soluble impurities, although these proteins were made as pure as the science of chemistry can now make them.

Keratin, fibrin, aleuronat and grain gluten are also eaten by amebas; the first two occasionally, the last two frequently.

2. Isolated proteins are sometimes ingested in food cups of varying size, sometimes without the formation of food cups. In the latter case the protoplasm merely flows around the food particle. Occasionally a food cup is started before the ameba comes into physical contact with the protein particle.

3. Granular amebas eat isolated proteins much more frequently than the raptorial, and they retain what they eat much more readily.

4. It cannot be stated what qualities in these proteins induce ingestion. The fact that the one which is probably the most readily purified, zein, is not eaten, may indicate that perhaps minute traces of soluble material are present in globulin, lactalbumin and ovalbumin. It is also possible that these proteins are very slightly soluble in the water in which amebas live.

5. From the point of view of general behavior several facts are brought to light.

a. An ameba utilizes the vestige of a former pseudopod for the projection of a new one whenever it is possible rather than form an entirely new pseudopod. The reactions of an ameba at a given time are, in a definite manner and to a large extent, conditioned by its behavior, that is, by changes in the shape of its body, during the preceding minutes.

b. There is a large amount of objective evidence that positive and negative tendencies of reaction with respect to a single source of stimulation are effectively present in ameba, and that reactions are not due in any sense to the direct effect of the stimulus.

POSTSCRIPT

After this paper was in manuscript the species reference of 'raptorial' and 'granular' amebas was investigated. The granular amebas were found to be of two species: Amaeba proteus Pallas emend. Leidy, and A. discoides Schaeffer; the raptorial of one species A. debia Schaeffer. See my paper in Science, September, 1916 for descriptions of these species.

EXPLANATION OF PLATES

The figures are camera lucida drawings taken from the laboratory notes without alterations. They are all of the same magnification. A scale by means of which the size of the amebas and of the test substances can be estimated is shown on plate 3.

The figures are numbered serially for reference. The numbers are placed inside the figures whenever possible. They are to be looked upon as labels only. They have no other significance. An x following a number, as 411x, indicates the end of the experiment illustrated by figures 408 to 411x inclusive. A new experiment starts with figure 412 and ends with figure 432x, and so on. A number with xx following it indicates that the next experiment was performed upon a different ameba. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed on any one ameba. The figures were drawn in vertical columns whenever possible. The work on the various amebas is not arranged in strict chronological order. The given arrangement was decided upon in order that the experiments on a particular problem could be presented together. Occasionally a figure as drawn in the laboratory records is left out in these plates. Thus figure 428 does not appear in the experiment illustrated by figures 412 to 432x because it does not add anything of importance to the understanding of the experiment. Other figures were omitted for similar reasons.

The time of the beginning and the end of each experiment is given in hours and minutes. In many cases the time of drawing of each figure is also given, and where it is not given it may easily be computed, as the time of all figures where it is not indicated are spaced equally in time, in any one experiment.

The arrows show the direction of active protoplasmic streaming. The arrow in the last figure of each experiment denotes the direction the ameba took in moving away when observations were terminated.

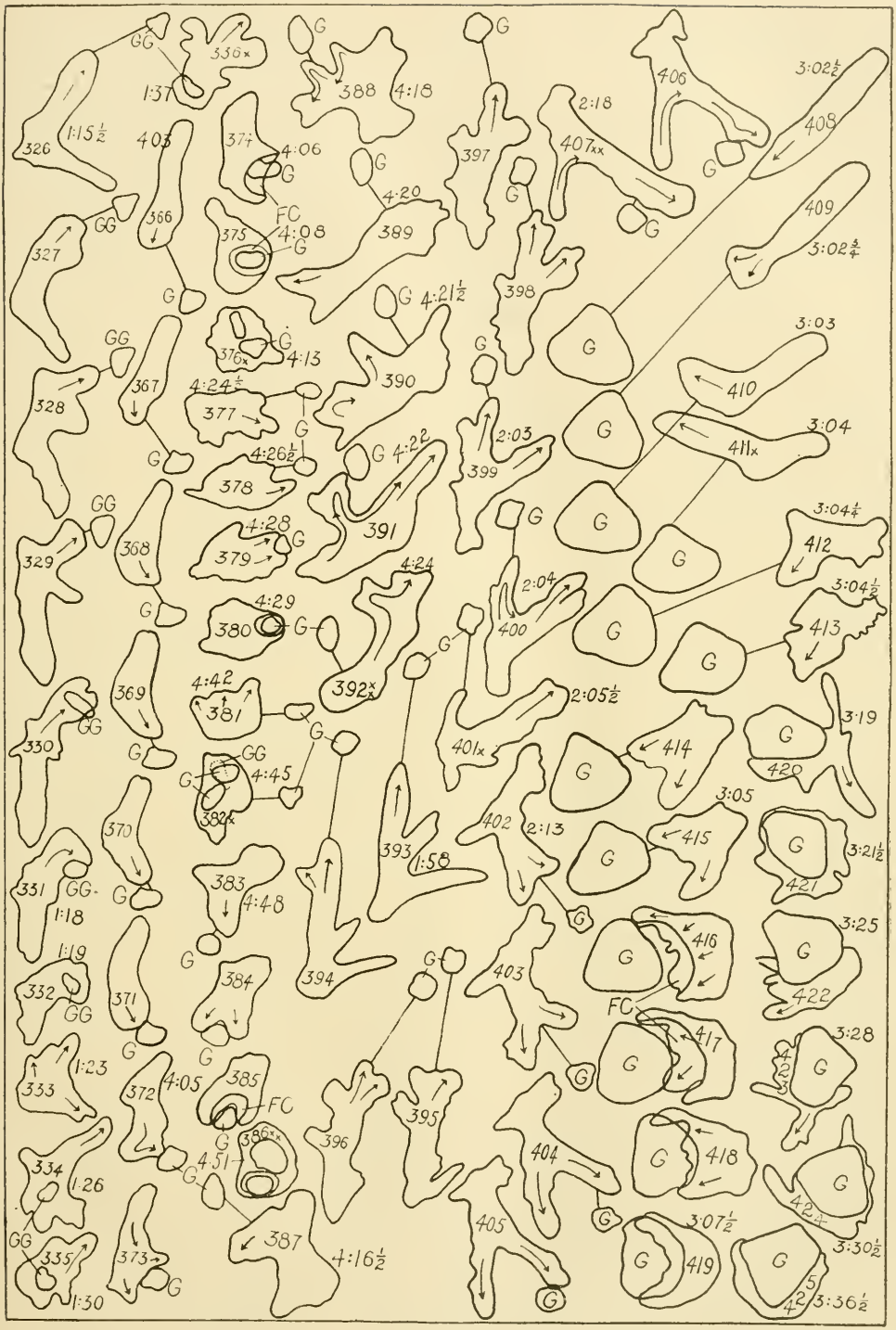
All the work was done facing a north window. All the figures were drawn in the same position in the laboratory and on the plates. The top of each plate therefore points toward the North.

It will be noted that there are slight differences in the size and shape of the same test object as drawn in the figures of any single experiment, even if the object was not rolled around by the ameba. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule, the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of the figures see the text.

The test objects are labeled in abbreviated form as follows: (For quick and correct reference the test objects are connected with the proper ameba by leader lines; these lines have no other significance.)

A, aleuronat
F, fibrin
FC, food cup
G, globulin
GG, grain gluten
K, keratin
L, lactalbumin
O, ovalbumin
Z, zein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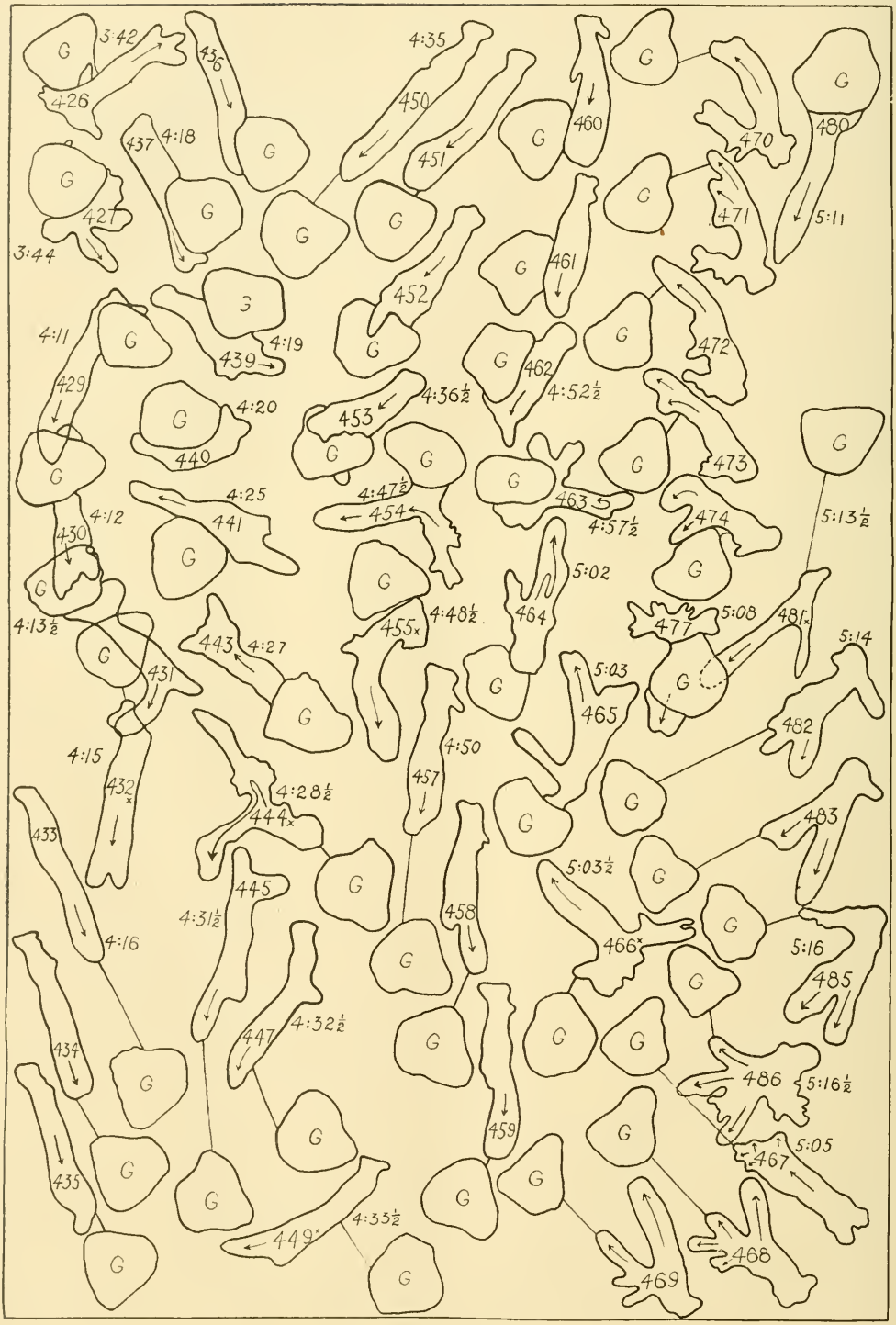
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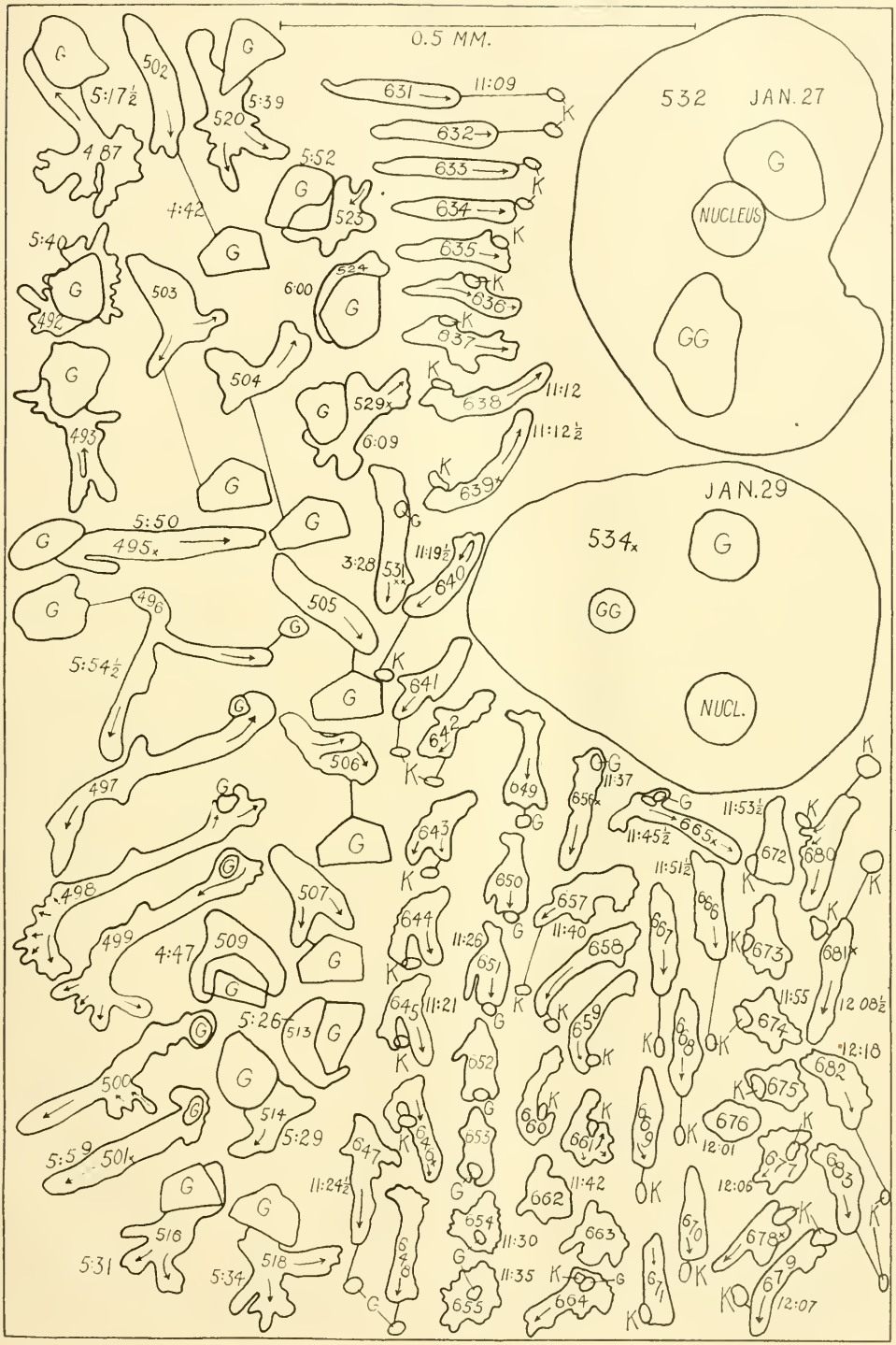
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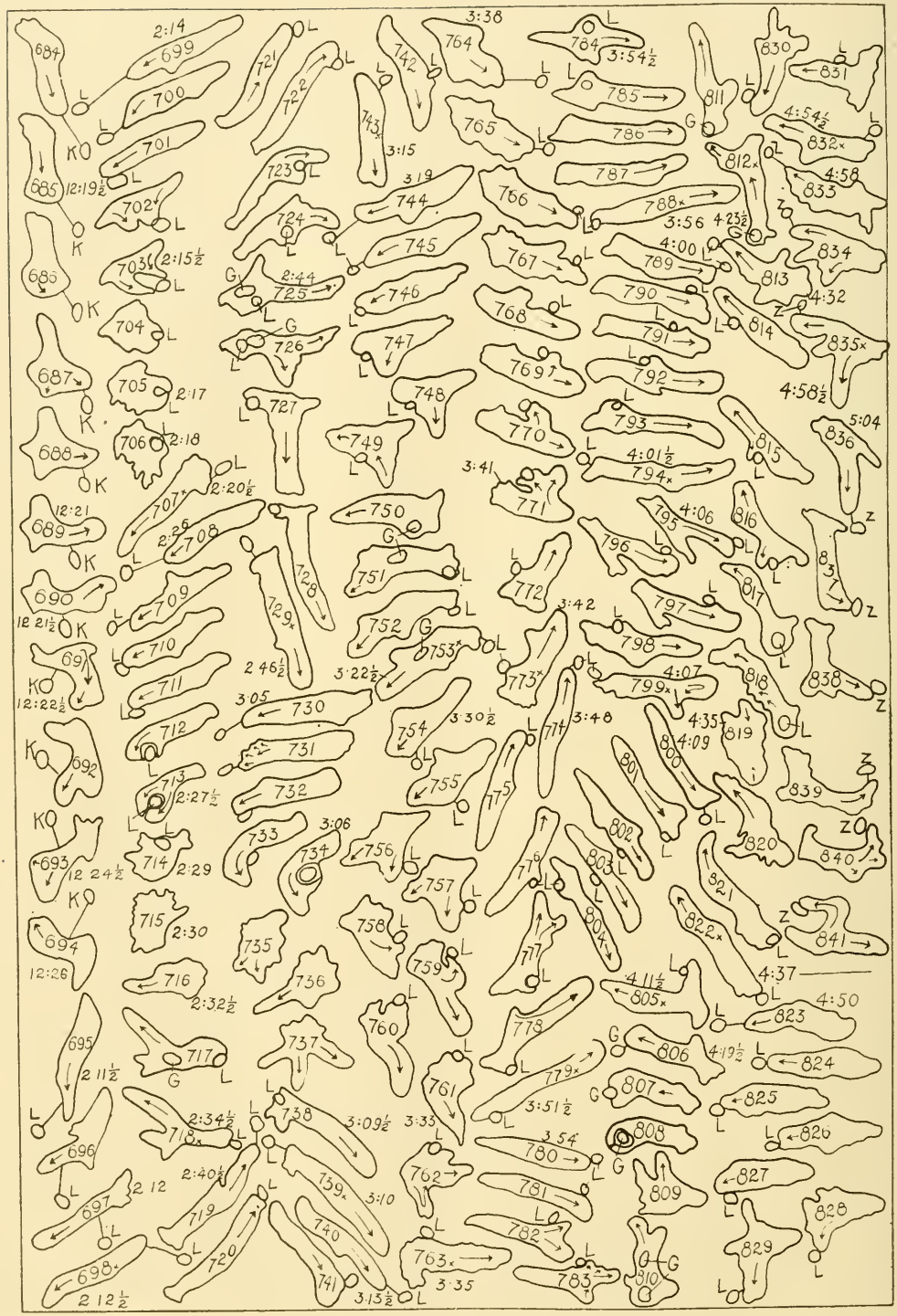

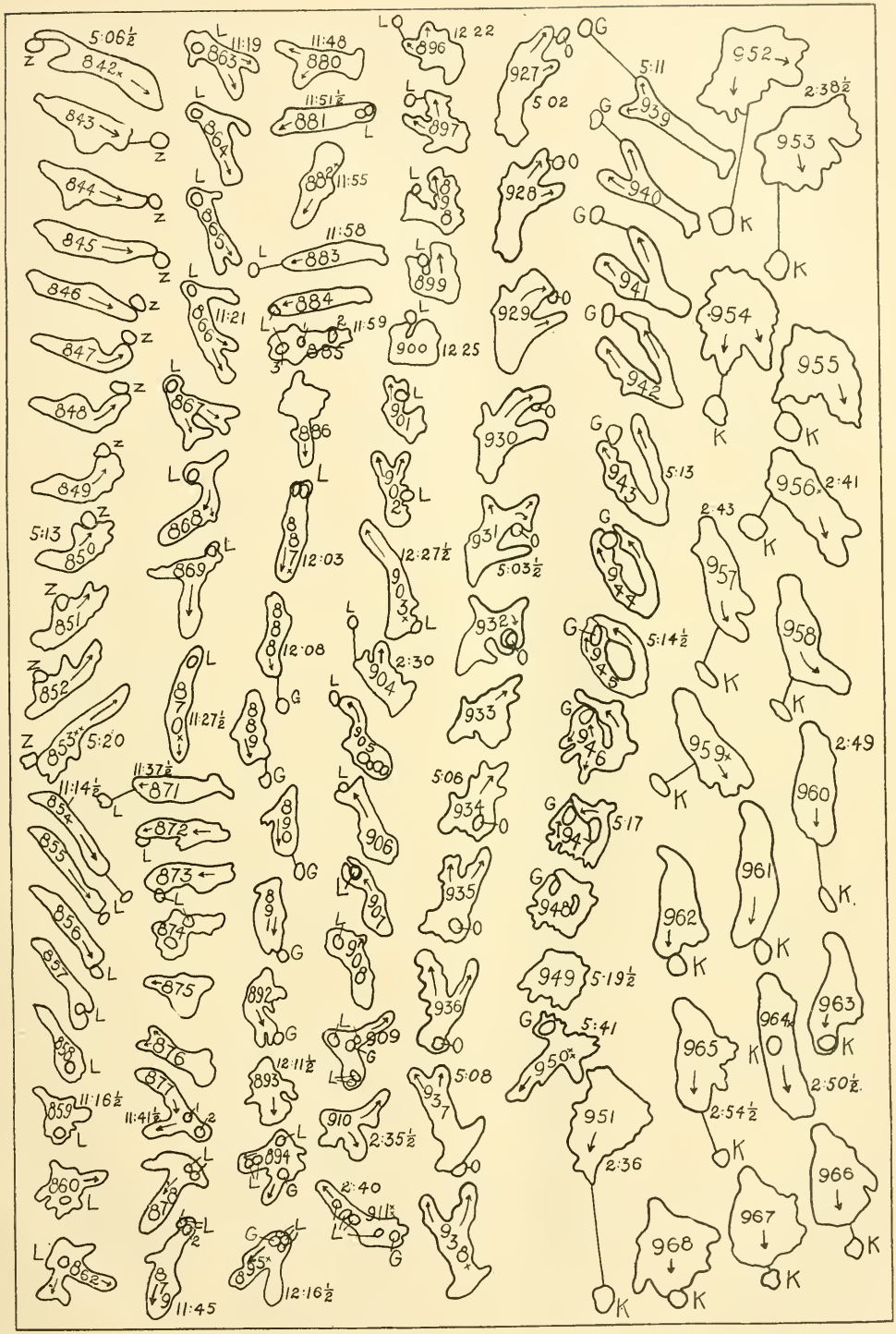
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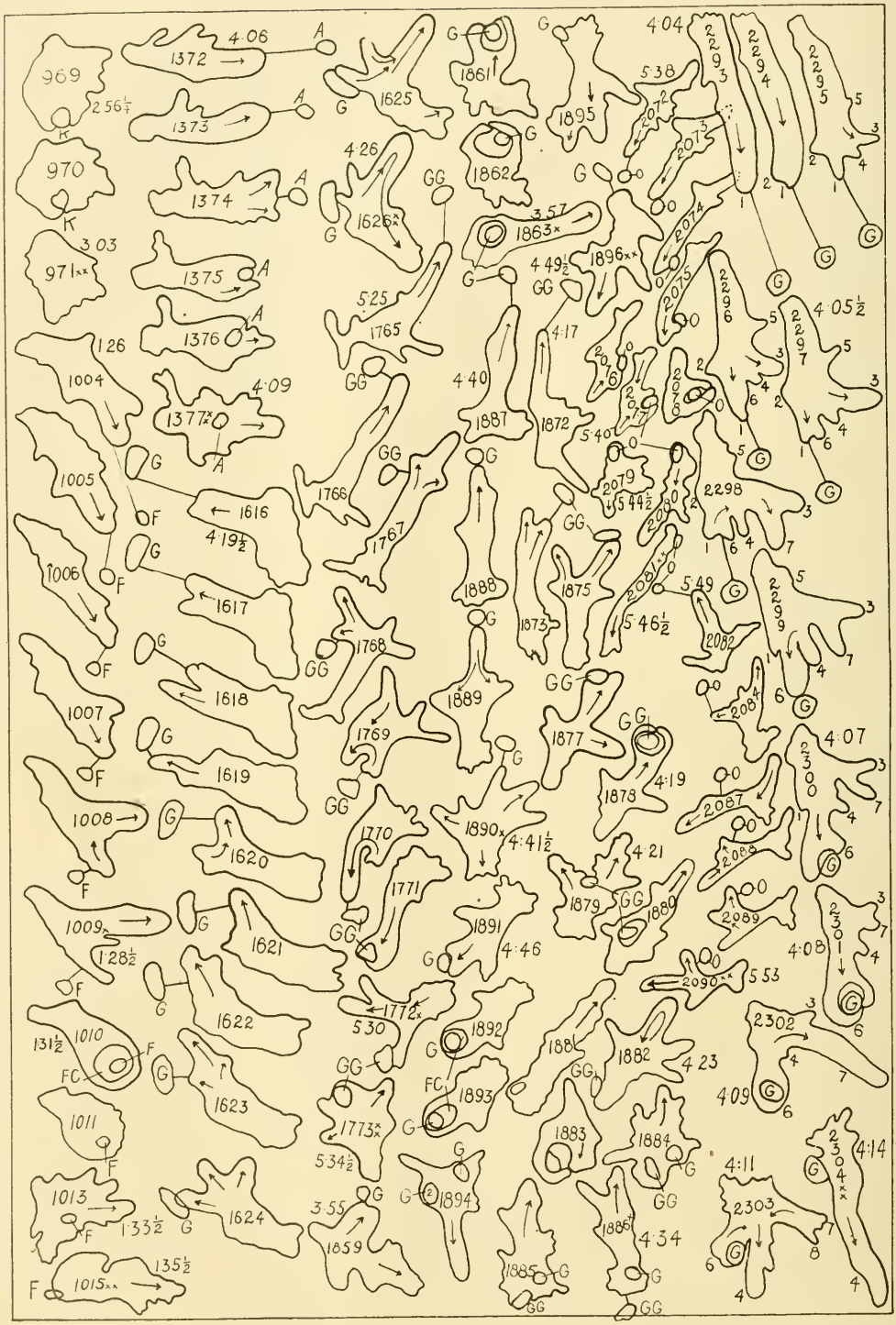
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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 284.

NERVOUS TRANSMISSION IN THE ACTINIANS

G. H. PARKER

THREE FIGURES

Experimental studies of the direction of nervous transmission in the discs of medusae have been made by many investigators since the days of Eimer ('78) and Romanes ('85), but very little work of this kind has been done on polyps. The following observations were made on medium-sized specimens of Metridium marginatum Milne-Edwards with the view of ascertaining something of the course of transmission in these animals.

Perhaps the most usual response that Metridium and other like actinians show is the retraction of the oral disc, and this response has been made the chief basis of the following research. It can be called forth easily by mechanical stimulation and it serves as a reasonably good test for the sensitiveness of different parts of the actinian's body to this form of stimulus. An exploration of the surface of Metridium by means of a delicate, blunt glass rod showed degrees of sensitiveness in various regions as follows:

A. Almost insensitive to contact with the rod were the general surface of the pedal disc, the lips, and the esophagus.

B. Only very slightly sensitive were the column between the oral disc and the sphincter, the intermediate zone of the oral disc (the space between the tentacles and the lips), and the surface of the siphonoglyph.

C. Slightly sensitive were the tentacles and the equatorial portion of the column.

D. Fairly sensitive was the surface of the column in the neighborhood of the sphincter.

E. Most sensitive was the surface of the column near its pedal margin, a fact already pointed out for other species by Fleure and Walton ('07, p. 213).

From all the regions named in the preceding statements—and these regions together represent practically the whole outer surface of the actinian—the retraction of the oral disc could be called forth by mechanical stimulation of varying degrees of intensity. It will be recalled that, according to the scheme of nervous transmission in actinians proposed by the Hertwigs ('79–80), a scheme based exclusively on histological evidence, impulses from the ectoderm of the animal were supposed to pass to the oral disc, thence down the ectoderm of the esophagus to the mesenteric filaments and thus to the entodermic musculature, such as the longitudinal muscles of the mesenteries. To ascertain the course of these impulses as tested physiologically, a series of observations were made on actinians that had previously been subjected to operations designed to interrupt certain possible courses.

1. If the wall of the column of Metridium is cut through in a complete ring equatorially, that is, the column is girdled, a mechanical stimulus applied either to the oral portion of the column wall or to the pedal portion will call forth a general retraction of the oral disc.

2. If the whole oral disc is cut off, a mechanical stimulus applied near the former equator or, better, near the pedal edge, is followed by a contraction of such parts of the longitudinal mesenteric muscles as remain in the preparation. This experiment confirms the statements made by Jordan ('08) that the oral disc is not necessary for the activity of the longitudinal mesenteric muscles, and it shows that, contrary to the opinion held by the Hertwigs ('79–80), there are direct transmission paths from ectodermic sense cells to entodermic muscles independent of the esophagus. Histological evidence of such paths has already been advanced by von Heider ('77, '79, '95), Havet ('01) and Parker and Titus ('16).

3. If a tongue of the column wall is cut from the pedal edge of the column up to its equatorial region and there left in organic connections with the rest of the animal (fig. 1) and a mechanical stimulus is applied to the free end of the tongue (X), a response of the whole system of longitudinal mesenteric muscles follows.

4. If a similar tongue is cut from the oral edge of the column down to its equator, a stimulus applied to the free end of the tongue will again call forth a retraction of the oral disc.

5. If a tongue of tissue is cut equatorially from the column so as to girdle it for half its circumference, and a stimulus is applied to the free end of the tongue, such stimulus is only occasionally followed by a retraction of the disc, showing that this least sensitive part of the column is not in free nervous connection horizontally with the rest of the column.

6. If the pedal edge of the column is cut off by an incision parallel to this edge and about 3 mm. inside it, thus producing

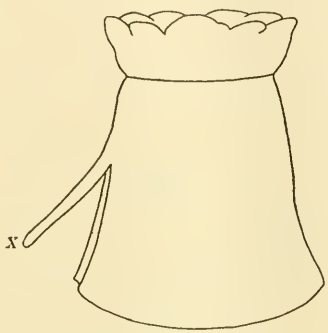

Fig. 1 Side view of a Metridium from which a lateral tongue of tissue has been cut (Experiment 3); *X*, region of stimulation.

a band of tissue 4 to 5 cm. long and attached by an end only to the animal (fig. 2), a mechanical stimulus applied to the free end (*X*) of this band is followed immediately by the retraction of the oral disc. If this band is anywhere completely cut across, a stimulus distal to the cut is never followed by a response of the disc even though the two faces of the cut are in contact.

7. If a Metridium is cut through vertically so as to separate it completely into two parts except for the pedal disc, the stimulation of one part is followed by a retraction of the halves of the oral disc in both parts, showing that the pedal disc is a means of transverse nervous connection for the animal.

8. If a Metridium is cut vertically in two except for a small connecting bridge near the pedal edge of the column, the mechanical stimulation of the column of one part is followed by oral-disc retractions in both parts.

9. If a Metridium is cut through vertically except for its oral disc, the mechanical stimulation of the column of one part is followed by a contraction of the longitudinal mesenteric muscles of both parts, thus demonstrating transverse nervous connections in the oral disc.

10. If the column of a large Metridium with a pedal disc over 10 cm. in diameter is cut through in an oblong outline, 4 cm. by 2 cm. (fig. 3), a superficial piece of the column results that is

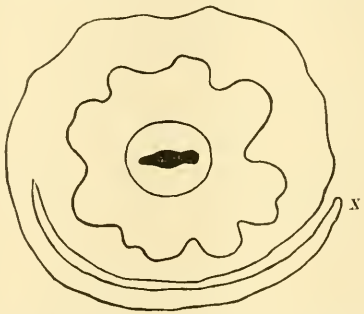

FIG. 2 Oral view of a Metridium from which an edge of tissue has been cut (Experiment 6); *X*, region of stimulation.

attached to the rest of the animal only through its mesenteries. Nevertheless when the middle of this piece (*X*) is stimulated mechanically or by discharging on it a small amount of hydrochloric acid in sea-water, a withdrawal of the whole oral disc follows. This response ceases when all the organic connections of the piece with the rest of the animal are severed by cutting through the attached mesenteries, thus allowing the piece simply to lie in place. The cessation of response under these circumstances shows that the transmission must be nervous and not due either to the mechanical effects of the contraction of the piece itself on the rest of the animal, nor to an accidental overflow of acid.

In all the experiments thus far described the responses of the longitudinal mesenteric muscles followed quickly the application of the stimuli, certainly in less than a second. There seems therefore to be no doubt that the transmission is a true nervous operation. Moreover, as the position of the application of the stimuli and the direction of the cuts show, the pedal disc, the column, and the oral disc must contain nervous connections of a net-like character by which the ectodermic surfaces are put into direct connection with the longitudinal mesenteric muscles. There is further the strongest kind of physiological evidence for nervous connections from the ectoderm directly through the

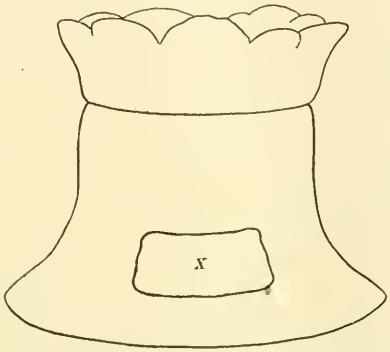

Fig. 3 Side view of a Metridium on which an oblong outline has been cut through the column wall (Experiment 10); *X*, region of stimulation.

mesogloea to the entoderm. Of the regions thus far mentioned the one in which these nervous connections are least developed is the equator of the column.

11. If a Metridium is cut vertically in two so that the resultant parts are connected only by the lips, not even the esophagus or the oral disc remaining intact, and a mechanical stimulus is applied to the column of one part, the portion of the oral disc in that part regularly retracts, that in the other usually does not. This form of experiment was repeated many times and with almost invariable results; it was only very rarely that the application of a stimulus to the column of one side was followed by a response from the longitudinal muscles of the other side. It,

therefore, seems certain that the lips are at best poor means of nervous transmission from one half of the body to the other. Thus it appears that the scheme of nervous transmission proposed by the Hertwigs ('79–80) and accepted with slight modifications by Wolff ('04) and by Grošelj ('09) fails in two fundamental particulars; first, it does not take into account direct connections between the outer ectoderm, particularly that of the column, and the deep-lying entodermic muscles, and, secondly, such parts as the lips, which, according to this scheme, are an essential portion of the connection between the ectodermic and entodermic nervous tracts, prove on examination to have almost no capacity as nervous conducting organs. For these reasons, as well as for the facts advanced in the preceding account, I regard the scheme for nervous transmission advanced by the Hertwigs as essentially untenable. Nervous transmission in actinians is accomplished in large part over tracts that penetrate the mesogloea and is in no sense strictly limited to the basal parts of the ectoderm and the entoderm.

Such a plan, however, must not be interpreted as one of purely diffuse transmission. There is ample evidence in certain parts of Metridium at least of specialized transmission. If the juice from a crushed mussel (Mytilus edulis) is discharged on the pedal disc or the column of Metridium, no response follows. If on the other hand the juice is discharged on the tentacles, it will induce the characteristic feeding movement of these organs and a wide gaping of the esophagus. When discharged on the lips, it is also followed by a wide opening of the esophagus. If a solution of hydrochloric acid $\frac{n}{2}$ in sea water is applied to the column or to the tentacles, a retraction of the oral disc follows; but if it is applied to the lips, a widening of the esophagus takes place. Thus a stimulation of the tentacles by hydrochloric acid induces a contraction of the longitudinal mesenteric muscles and by mussel juice a contraction of the transverse mesenteric muscles. Such diverse reactions from the same receptors must involve a certain amount of internal differentiation in the transmission mechanism which to my mind foreshadows in the primitive neuromuscular mechanism of such animals as Metridium the

differentiated transmission tracts of the higher animals. Whether this differentiation takes the form of semi-independent nerve nets, as suggested by von Uexküll ('09), or is of the nature of somewhat isolated and, in consequence, nerve-like tracts which connect one semicenter with another, cannot be stated.

SUMMARY

1. Nervous transmission may be accomplished from almost any part of the ectoderm of Metridium to its longitudinal mesenteric muscles.

2. Experiments in which the receptive part of a Metridium is connected with the effector portion by only a small bridge of tissue demonstrate that these nervous connections occur in such a variety of positions as to call for the assumption of a nerve net.

3. These connections in many places pass directly from the ectoderm, through the mesogloea, to the entoderm. In connecting the ectodermic with the entodermic systems the lips, and probably the esophagus, are not as important organs as many other parts of the body.

4. Notwithstanding the generally diffuse condition of the transmission system in Metridium, there is evidence also for a certain degree of specialization in this system. Stimulation of the tentacles by mussel juice calls forth a gaping of the esophagus (contraction of the transverse mesenteric muscles) and by weak hydrochloric acid a retraction of the oral disc (contraction of the longitudinal mesenteric muscles). These two forms of response afford good ground not only for the assumption of independent receptors but for the belief in relatively independent transmission tracts, a first step in the kind of differentiation so characteristic of the nervous organization in the higher animals.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 285.

THE MOVEMENTS OF THE TENTACLES IN ACTINIANS[1]

G. H. PARKER

ONE FIGURE

The tentacles of actinians have long been declared to be capable of carrying out many of their normal activities even after they have been cut from the body of the polyp to which they belong. This peculiarity, which was recorded apparently first by von Heider ('79, p. 248) and has since been noted by others (Parker, '96; Wolff, '04; Chester, '12), has been accepted as evidence that each tentacle contained a neuromuscular mechanism sufficient for its own activity and that it is therefore not dependent upon the nervous control of other parts of the animal's body for the production of those movements that it ordinarily exhibits. To test the validity of this view, which has recently been questioned by Rand ('15, p. 208), was the object of the studies recorded on the following pages.

The work was carried out on Metridium marginatum Milne-Edwards, and Sagartia luciae Verrill at Woods Hole, Massachusetts, and on Condylactis passiflora Duch. and Mich. at Bermuda. The advantage of Condylactis for this kind of work is evident because of the large size of its tentacles, and almost all of the experiments recorded on the following pages were performed on this species.

In a full-grown Condylactis the oral disc may measure as much as 12 cm. in diameter and carry as many as a hundred tentacles. About half of these are near the outer edge of the disc and the remainder are scattered toward the mouth. The mouth is central in position, usually diglyphic, and partly covered by folds of the peristome.

Each tentacle is from 12 to 15 cm. long, with a diameter of about 1.5 cm. at its base and terminates distally in a blunt end in which there is a pore. The tentacles are light brown in color

[1] Contributions from the Bermuda Biological Station for Research. No. 54.

with whitish markings; in some specimens they are tipped with pink. They are at most sparsely ciliated and provided with only a few nematocysts; their walls are thin. When cut they may contract from a length of 15 cm. to 1.5 cm.

If an expanded quiescent tentacle is touched near the tip with a silver sound or a glass rod, the tentacle contracts, usually bending toward the stimulated side. The tentacle often sticks to the object with which it is touched and it may in contracting thus exert a considerable pull, showing that its surface is remarkably adhesive and that its musculature is vigorous. This adhesiveness is especially noteworthy in specimens with pink-tipped tentacles and these individuals are known to adhere with the pedal disc to the glass wall of an aquarium more tenaciously than those without such coloration.

If a strong stimulus is carefully given to a tentacle not closely surrounded by others, the tentacle may contract without touching the others and yet these may also respond, thus giving evidence of basal transmission from tentacle to tentacle. A tentacle, if only once slightly touched, usually quickly returns to its former position and quiescence.

If a piece of crab-meat or fish-flesh is brought in contact with a tentacle, it adheres firmly to the tentacle which quickly contracts and is usually covered by several adjacent tentacles. The piece of flesh is thus held on the disc while the mouth gradually moves toward it and on reaching it swallows it. The animal then slowly returns to a state of quiescent expansion.

If a tentacle is cut off at almost any level, the stump at first contracts but after a time again elongates to its appropriate length, its cut end being closed by a nipple-shaped pucker (Rand, '09, '15). The portion of the tentacle cut off, also contracts and will remain indefinitely so at about one-third its original length. Such a fragment of tentacle will live in seawater for several days.

In specimens of Condylactis under natural conditions tentacles are often seen marked with regions of pronounced constriction. The portion of the tentacle distal to the constriction has been often observed to be cast off and it is probable that these con-

strictions are the first step in a process of tentacular autotomy that seems to be characteristic of Condylactis.

In an experimental study of the tentacles, it is necessry among other things to cut off the tentacles from the body of the polyp and to study them thus isolated. When a tentacle is treated in this manner, it contracts, as already stated, to about one-third its former length and it will remain in this condition alive in sea-water many days. At the moment the connection of the tentacle with the actinian's body is severed its fluid content in part escapes and water flows out of the polyp from the hole left on the stump, thus showing that the tentacle as well as the animal is distended under slight pressure. It might, therefore be assumed that the full expansion of the tentacle was due to this slight pressure and that, when the tentacle was cut off, the partial contraction that followed was due to the release of the tentacle from internal pressure.

To test this hypothesis a tentacle, after having been cut off from the polyp and allowed to come to as full distension as it would, was tied to the end of a glass tube preparatory to distending it further with sea water. On binding it to the tube it contracted vigorously to only a small fraction of its former length and remained thus for hours. Sea-water was finally run into it through the attached tube and when this water stood at a height of 8 to 10 cm. in the tube, the tentacle without expanding began to allow the contained fluid to escape through its terminal pore. This was then tied off and the pressure in the tentacle was increased by running more water into the tube. At about 14 to 15 cm. of pressure the tentacle without having expanded ruptured on the side and with the escape of the water it contracted completely. During all of this experiment the tentacle was rigid and tight and very unlike the normal tentacle, which when touched gently or moved by water currents is evidently under internal pressure but pressure of only a very slight amount, probably not more than that of a few millimeters of water. It is clear from these observations that the pressure of sea-water as applied in this experiment is not a means of restoring a severed tentacle to a normal state and that the further contraction that

often appears in a partly contracted tentacle as the internal pressure increases shows that the applied pressure itself is a stimulus to contraction (Rand, '09, p. 206).

If then, the contraction of a severed tentacle is not due, simply to a release from pressure, it may depend upon some influence emanating from the stump of the tentacle or from the polyp as a whole, which influence on being interrupted by the cut allows the tonus of the tentacular muscles to exert itself unrestrainedly. To test this hypothesis, tentacles were partly cut through about midway their length and then allowed to come to a condition of quiescence. Such tentacles became normally expanded and elongated proximal to the cut but were somewhat contracted distal to it, the cut itself exhibiting a well marked lateral pucker. Such tentacles, if stimulated vigorously at the base, would exhibit longitudinal contractions not only in the basal portions but also to some extent in the portions distal to the wound. It thus appears that the contraction of the distal part of a tentacle is not overcome even through the connection of this part with the proximal part by a bridge of tissue capable of transmitting impulses from the polyp or basal part of the tentacle to the peripheral part of this organ.

If the contraction of the severed tentacle is not due to loss of pressure, or loss of a proximally located influence inhibiting tentacular tonus, it is most probably due to the influence of the cut itself. It is well known that wherever an actinian is injured the muscular tonus of the given region is greatly increased and it is therefore not surprising that on cutting off a tentacle the whole organ should contract in a more or less permanent way. That this is the probable explanation of the partial contraction of a severed tentacle is seen from the fact that if such a tentacle is firmly tied at its base, whereby its injury is considerbly increased, it becomes permanently still more contracted in that the tonus of its neuromuscular mechanism is appreciably increased.

On the assumption that the partial contraction of the severed tentacle had an operative source, I tried to avoid this difficulty by anesthetizing the base of a tentacle so as to obtain a non-responsive part to cut and tie and leave the greater part still

responsive. But I failed in devising any technique by which the root of the tentacle could be rendered insensitive and the distal part left unaffected.

I therefore turned to methods of procedure that were least disturbing to the normal tentacles. The best of these consisted in holding at the surface of the sea-water a quiescent severed tentacle by means of a minute hook made by bending slightly the pointed end of an entomological pin (fig. 1). Into the open end of such a suspended tentacle sea-water could be run from a glass pipette and thus the tentacle could be brought to a reason-

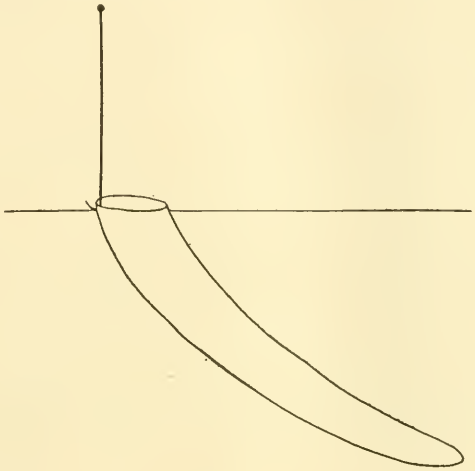

Fig. 1

able degree of distension. Such severed tentacles when first put on the hook were contracted to about one-third their normal length. As their condition did not differ essentially from that of loosely floating severed tentacles, I concluded that the effect of the hook was negligible. On discharging water into them they gradually expanded till they were about two-thirds as long as they were before their separation from the polyp. They exhibited moreover just about that degree of distension and mobility that was seen in the attached tentacles. If, now, more water was discharged into them, they were very likely to elongate a little and then contract considerably discharging much of the contained water. This response confirms the opinion already

expressed in this paper that an increase of internal pressure beyond a certain point will call forth a vigorous muscular contraction. If, however, this mild pressure is not exceeded, a tentacle expanded to about two-thirds its natural length and exhibition its normal pliability is readily obtained.

The response of the tentacles to internal pressure is probably not without its significance in the general reactions of actinians. When a sea-anemone contracts vigorously, its fluid contents are put under much increase of pressure and this increase must be almost instantly transmitted to the contents of each tentacle, thus stimulating it to contraction. In this way the whole circle of tentacles might easily be brought to general withdrawal through the individual response of the neuromuscular mechanism of each tentacle acted upon by a common purely physical factor. The ease with which such a controlling factor could be confused with nervous influence must be apparent.

Having obtained in isolated tentacles a close approximation to the resting condition of the attached tentacles, it remained to compare the reactions of these two classes. Suspended tentacles filled to about two-thirds their natural length will remain quiescent in sea-water for a considerable period. From time to time, however, they show spontaneous movements consisting of slight contractions and twistings by which more or less of their contained fluid will be discharged. If this is replaced they will reëxpand and thus periods of quiescence are followed by periods of spontaneous movements. In these respects the severed tentacles reproduce very closely the behavior of the normally attached tentacles.

If a suspended tentacle is gently touched it exhibits irregular reaching and writhing movements, whereby it will partly empty itself. After it has been refilled, a more vigorous stimulus will induce a more or less spiral contraction, in which state the tentacle will remain some minutes. Again it must be refilled. In the response both to the slight and to the vigorous mechanical stimulus the severed tentacle reproduces in a most striking way the movements of the attached tentacles under like stimulation.

When an attached tentacle is gently touched on one side midway its length, the tentacle as a whole contracts but without much bending. If it is touched on the tip, the response is mostly a terminal waving back and forth. If it is stimulated on one side near the base, the contraction is chiefly basal and on the stimulated side. These responses are reproduced quite clearly by isolated tentacles. Thus the responses of the two classes of tentacles to localized stimuli are strikingly similar.

If a small amount of one per cent acetic acid is discharged on an expanded severed tentacle, the tentacle contracts quickly with a curious appearance as though it were withering. After it has been washed with seawater, it will expand again in about three to four minutes. A second and a third response have been elicited from such tentacles and these responses reproduce most strikingly the movements of attached tentacles. To a tenth per cent acetic acid both classes of tentacles showed a slight local shortening. To a hundredth per cent they responded by a slight curving. To a thousandth per cent neither kind of tentacle showed any response whatever as was also the case when pure seawater was discharged on them from a pipette.

In none of my experiments on Condylactis did I get evidence of a specific contraction of the circular muscle fibers to stimulation by very weak acetic acid as von Uexküll ('09) found for Anemonia.

To rain water discharged on the tentacles from a small pipette, no noticeable response was made by either class, but to seawater containing the juice from a crushed mussel the attached tentacle exhibited active writhings often accompanied by elongation. It was remarkable how strikingly similar to these were the responses of the isolated tentacles to the same juice.

To a one per cent solution of quinine hydrochloride in rain water both classes of tentacles responded by quick contractions and often local constrictions. The solution was applied by a pipette to limited areas on the outside of the tentacles.

In all the tentacular reactions studied the responses of the isolated tentacles agreed most strikingly with those of the normally attached tentacles. Of course the reactions of the iso-

lated tentacles are not exact duplicates of those of the attached ones. They are feebler and less precise, but aside from these peculiarities they are so strikingly similar that their resemblance is the conspicuous feature of the operation. The differences between the two sets of reactions are due in my opinion to the partial contraction of the excised tentacles. This partial contraction, as already pointed out, seems to be purely operative in its source, and it is this, in my opinion, that is the cause of the lowered responsiveness rather than the absence of some extra-tentacular influence. That this explanation is correct, may be shown by stimulating unsevered tentacles that are already in a state of partial contraction due to a preceding stimulation. When such a tentacle is restimulated, it responds, as repeated trials have shown me, with just that partial vigor and lack of precision which characterize the isolated tentacles. I, therefore, believe that we have ample grounds for concluding that the slight differences in the reactions of isolated and attached tentacles are purely operative.

The tentacles of Condylactis, like those of most actinians, possess a longitudinal musculature in the ectoderm and a circular one in the entoderm and are supposed to exhibit a nervous layer with each muscular layer. The means of stimulation thus far used in these experiments have been applied to the ectoderm and the responses thus called forth have been chiefly in the longitudinal muscle of this layer, though the deeper circular muscle has undoubtedly also been generally involved. With tentacles of the size of those in Condylactis it is possible to apply stimuli to the entodermic surface as well as to the ectoderm and by this procedure some idea of the responsiveness of this surface can be obtained.

Into an isolated tentacle suspended in seawater by a hook and so held that its open end was well above the surface of the water, a small amount of one per cent acetic acid was injected. After about two seconds the tentacle gradually shortened. It was then thoroughly washed with seawater and rehung. When it had become reëxpanded, the discharge of a small amount of one per cent acetic acid on the ectodermic surface was followed by

an immediate shortening. These tests were repeated several times and always with the same outcome; a shortening after two or three seconds when the stimulus was applied to the interior of the tentacle and a sudden shortening when it was applied to the exterior. When mussel juice was substituted for acetic acid, a writhing response was produced in about four seconds by the application of this stimulus to the inside of the tentacles, but this response took place almost immediately when the juice was applied externally. Quinine hydrochloride in one per cent solution was immediately effective when applied to the exterior and called forth a response only after more than a minute, when applied internally.

It thus appears that stimuli which call forth specific reaction when applied to the ectoderm of the tentacles induce the same movements when they are applied to the entoderm, but only after a somewhat longer interval.

Is this difference in the rate of response a difference in the nervous activities of the ectoderm and the entoderm of the tentacle or is it to be accounted for by the direct stimulation of the ectoderm in the quick form of response and the less direct one in the slow form in that in the latter it requires an appreciable time for the stimulating fluids to pass from the cavity of the tentacle through its wall to the ectoderm? If the latter explanation is correct, it ought to be possible to get evidence of the penetration of the wall of the tentacle by such a solution as that of acetic acid.

To test this question an isolated tentacle was suspended in seawater and close to it was hung a piece of blue litmus paper; near at hand in the seawater was also suspended a piece of red litmus paper. Deep into the seawater filling the tentacle was now discharged about a drop of one per cent acetic acid. The tentacle contracted slightly but not enough to cause it to overflow. In about half a minute the blue piece of litmus paper began to redden, showing that the acid had transfused the living walls of the tentacle. Meanwhile the piece of red litmus paper had begun gradually to change blue, doubtless from the slight alkalinity of the seawater. In two other tests evidence of the pene-

tration of the wall by acid was obtained in 45 seconds and in 55 seconds.

To be certain that the reddening of the litmus paper was not due to any leakage through the pores at the end of the tentacle, the experiment was repeated with a tentacle the tip of which was tied. This brought about an extreme contraction of the tentacle. Nevertheless the transfusion of acid through the wall of the tentacle was observed in from two to two and a half minutes. The longer period needed for the transfusion in this instance, as compared with that in tentacles not tied at the tip, is doubtless due to the increased thickness of the wall of the tentacle in contraction. It therefore seems not improbable that the response of a tentacle to chemical stimuli discharged into its interior is not due to the stimulation of entodermic receptors but to the transfusion of the substances through the wall of the tentacle to the ectoderm, where a normal external stimulation probably takes place.

Another method of testing for an entodermic nervous system is as follows. It has already been stated that a suspended isolated tentacle will contract when a one per cent solution of quinine hydrochloride is discharged on it or into it; quickly in the former case, more slowly in the latter. If such a tentacle is bathed externally in a preliminary way with a half per cent solution of cocaine hydrochloride, after five to eight minutes it will no longer respond to the quinine solution. If now this solution is at once discharged into the interior of the tentacle there is likewise no response. Thus the tentacular entoderm does not appear to be a receptive surface for internal stimuli, but merely transmits in a physical way substances which become effective stimuli only after they reach the ectoderm. The view that the tentacular entoderm is not a receptive surface and may contain no nervous tissue whatever, is supported by the fact that the muscles responding to all the stimuli applied to the interior of the tentacle are the distantly located longitudinal muscles, not the near-by circular muscle.

These various lines of evidence naturally raise the question whether there is after all in the entoderm of the actinian ten-

tacle any nervous system whatever. It has been pointed out elsewhere (Parker and Titus, '16) that the histological evidence for this layer is by no means conclusive and the physiological evidence as thus far elicted from Condylactis is entirely opposed to a belief in its presence. In only one point do I find anything in the physiology of the tentacular entoderm that is suggestive of a contained nervous system. It has already been observed by Rand ('09, '15) that when a tentacle of Condylactis is cut off, the stump closes through the action of the circular muscles, whereby a nipple is formed. This closure does not occur in tentacles anesthetized by chloretone. I have elsewhere shown that chloretone will not check the action of the muscles in the acontia, though it is very effective as a narcotizer for the neuromuscular mechanism proper. I therefore believe that in actinians it directly effects nervous action and that its influence on muscle alone must be of an indirect kind. The narcotization of a tentacle must abolish this indirect influence, though I cannot say whether or not this involves some slight nervous activity. Aside from this I have been unable to get any evidence that the entoderm of the tentacle of Condylactis contains nervous elements. If such elements are present, they must be of a very primitive kind and certainly much less effective than those in the ectoderm, for the circular muscle fibers never react quickly and seldom respond as a whole in the way that the longitudinal muscle does, but much more usually in a local fashion, whereby rings of constriction are formed as in tentacular autotomy and other such spincter-like activities.

A good example of the different action of these two muscles is seen in the following experiment. If a suspended tentacle filled in the usual way with seawater is vigorously stimulated mechanically at one point, the whole tentacle instantly contracts. On being refilled it reëxpands in a few minutes except for a marked circular constriction in the region stimulated; this then gradually disappears. Thus the quick action of the longitudinal muscle and the slow action of the circular muscle is well demonstrated. If, now, the experiment is repeated but

with the point to be stimulated first thoroughly narcotized by dropping upon it crystals of magnesium sulphate, the stimulus is followed by a pronounced ring-like constriction in the circular fibers but by hardly any contraction in the longitudinal muscles. For these reasons I regard the ectodermic musculature as under the control of nervous elements and the entodermic at least open to direct stimulation.

Another general feature of the tentacles is the polarity exhibited by these organs. Thus the tentacular cilia regularly beat from the base toward the tip of this organ and hence exhibit polarization, but this feature is better seen in the neuromuscular reaction of these parts. When the tip of a tentacle is vigorously stimulated the whole tentacle is likely to respond, but when a point lower down on the side of the tentacle is stimulated, the reaction is chiefly from this point proximally. In other words transmission is more readily accomplished from the tip toward the base of the tentacle than in the reverse direction. The same kind of evidence comes from the reparative steps in regeneration. If a tentacle is cut off, the stump contracts vigorously and on reëxpanding forms a terminal nipple; the cut face of the distal segment usually contracts only a little and seldom if ever closes the open wound. Yet, if such a distal piece is again cut crosswise, its proximal part contracts and eventually forms a nipple while the distal piece remains almost unaffected. The polarization thus exhibited is in the same direction as that which was shown by the reactions of the attached tentacle to mechanical stimulation.

If the tentacles of an animal which has been thoroughly anesthetized with chloretone are touched, no response whatever follows. If they are cut neither the proximal nor the distal parts contract but both remain flabbily open. In consequence of these conditions I believe that the polarity of the tentacle in so far as it is exhibited by the reaction just noted is of a nervous nature, for it disappears on applying a drug which eliminates nervous activity.

When the nervous structure in the tentacle of actinians is examined with the view of seeking some condition upon which

this form of polarity can be based, a most simple relation is discoverable. As Grošelj ('09, p. 290) has pointed out, the nerve fibrils that arise from the sense cells in the tentacles of actinians extend as a rule in a direction corresponding with the length of the tentacle. In Bunodes many of these cells are bipolar and in that case one fibril extends distally along the tentacle and the other proximally, but there are also in this actinian a goodly number of cells that are unipolar and in such instances the fibrils almost invariably extend toward the base of the tentacle. In Cerianthus almost all the sense cells in the tentacular ectoderm are unipolar and their fibrils run almost without exception toward the base of the tentacle. As these fibrils transmit impulses away from the cell bodies with which they are associated, it follows that in both these actinians transmission must be predominantly toward the base of the tentacle and that consequently the region of response would be largely proximal to the region of stimulation. This is what is to be seen in the neuromuscular reactions of most actinian tentacles and I, therefore, believe that the polarity of these organs, as evidenced in the processes just mentioned, is dependent upon the proximal direction taken by the sensory fibrils in these structures whereby the nervous impulses are led to flow predominantly toward the base of the tentacle (Rand, '09, p. 235). This anatomical interpretation of the polarity of the tentacle is supported by the observation made by Chester ('12, p. 468) that after two tentacles of Metridium have been grafted together base to base there is no change in their (ciliary or) muscular polarity.

Concerning the relation of the tentacle to the organization of the animal as a whole, the preceding discussion must make several points clear. In Condylactis the tentacle is filled with fluid, whose slight pressure is dependent upon the activity of the body as a whole. It also receives from other parts of the animal through its base nervous impulses by which its neuromuscular mechanism may be set in operation, though this process is much more generally accomplished by the direct stimulation of the tentacle itself. Aside from the supply of nutriment transmitted to the tentacle from the general store produced in the gastrovas-

cular cavity of the animal (Jordan, '07), the transmitted pressure and the moderate inflow of nervous impulses are the only significant contributions of the whole animal to the tentacle. Yet, as the experiments already described show, neither of these are really essential to the responses of the tentacle, whose activities can be carried out by its own neuromuscular mechanism. How striking this condition is, can be made plain if the coördinated efficiency of an actinian's tentacle after separation from the body is compared with the state of an amputated appendage from an arthropod or a vertebrate. In the latter practically all traces of spontaneous movement and coördination have disappeared and only spasmodic jerks can be called forth by vigorous stimulation. Whereas in the former the range of response and the coördination of the individual acts differ from those of a normal animal, as has already been shown, only through slight operative disturbances. In seeking among the organs of the higher forms for a parallel to the actinian tentacle, one is continually reminded of the vertebrate heart, whose essential activity is so slightly interfered with even by removal from the body. The similarity in the action of the heart and of the tentacle under these circumstances is due to the fact that each possesses within its own substance a neuromuscular mechanism essentially independent of the rest of the body. The same is true of the separate arms of certain starfishes, which may move about as independent organisms before they have regenerated their deficiencies. Nor is the tentacle of the sea-anemone the only organ in this animal that exhibits organic independence of the kind already described. As will be shown in a subsequent paper, the pedal disc of the actinian when separated from the oral disc may creep about for a long time with well coördinated locomotor waves. This example, as well as others that might be cited from the actinians, as for instance the acontia, show the great independence of the parts of these animals in contrast with those in most other forms. To be sure, an actinian tentacle has never been known to regenerate a new individual and in this respect the tentacle is a totally subordinate organ, but in the direction already indicated it exhibits most remarkable independence and

though this question is obviously a relative one, when the activities of an isolated actinian tentacle are compared with those of the separate appendage of a higher animal there seems to me not the least ground for the opposition raised to the view of the high degree of organic independence of the tentacles from the rest of the body of actinians.

SUMMARY

1. The severed tentacles of Condylactis may be suspended in seawater with least disturbance to themselves by means of a small metal hook.

2. Under such circumstances they can be inflated by running water into them till they have attained about two-thirds their natural length. In this condition they are under a fluid pressure very nearly that which was natural to them and not greater than a few millimeters of water.

3. If this internal pressure is increased much beyond that at which the tentacle expands to about two-thirds its former length, the tentacle will contract vigorously and discharge much of the contained seawater.

4. The slightly contracted state of the expanded, excised tentacle is not due to lack of pressure, nor to the absence of inhibitory influences from the rest of the polyp, but to the cut at its base, which increases the tonicity of its neuromuscular mechanism.

5. Excised tentacles when stimulated by mechanical means, by food, or by chemicals react in essentially the same way as attached tentacles do. Excised tentacles are feebler and less precise in their reactions than attached tentacles are, a difference due to their partly contracted state, which in turn is dependent upon operative complications.

6. Stimuli when applied to the ectoderm of a tentacle are followed quickly by a muscular response; when applied to the entoderm they are followed slowly by the same form of response. This difference is due to the fact that the entodermal surface is not receptive and that stimulating substances applied to that

surface make their way only slowly through the wall to the receptive ectoderm, an operation which can be demonstrated in the case of acetic acid.

7. If there is a nervous layer in the entoderm of the tentacles of Condylactis, it must be extremely simple in structure and function as compared with that in the ectoderm.

8. The tentacles of Condylactis through their reactions exhibit marked polarity, ciliary and neuromuscular. The neuromuscular polarity disappears on narcotisation with chloretone. It is probably dependent upon the direction taken by the nerve fibrils which emerge from the sense cells. Where this direction has been determined, it is predominantly toward the proximal end of the tentacle.

9. The actinian tentacle, in contradistinction to such appendages as those of the arthropods or the vertebrates, contains a complete neuromuscular mechanism by which its responses can be carried out quite independently of the rest of the polyp.

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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 286.

PEDAL LOCOMOTION IN ACTINIANS[1]

G. H. PARKER

ONE FIGURE

I. INTRODUCTION

That many actinians can creep with more or less activity by means of their pedal discs over the surfaces to which they have attached themselves, seems to have been known to the earliest students of these animals, but aside from brief references to the simple fact of creeping the literature contains very little concerning this activity. The chief exception to this statement is the short paper by McClendon ('06), in which the creeping of Metridium marginatum is briefly described, and the notes made by Osburn ('14) on the distances covered in creeping by this species and by Sagartia leucolena. It is the object of the present paper to take up this activity more fully than has been done heretofore and to show in what respects it is related to actinian organization.

The work was carried out for the most part on four species of actinians: Metridium marginatum Milne-Edw. and Sagartia luciae Verrill at Woods Hole, Massachusetts; and Condylactis passiflora Duch. and Mich., and Actinia bermudensis Verrill at Bermuda. I am under obligations to Dr. P. H. Mitchell, Director of the Laboratory of the United States Fisheries Biological Station at Woods Hole, and to Dr. E. L. Mark, Director of the Bermuda Biological Station, for many courtesies shown me while I was working at these stations.

In dealing with the subject of pedal locomotion it was my intention to find out how the direction of such locomotion is related to the axes of the actinians, what the mechanics of the locomotion is, and what structural conditions are present in the actinians whereby this form of locomotion is made possible. Of the three fundamental and mutually perpendicular axes of

[1] Contribution from the Bermuda Biological Station for Research. No. 55.

this organism, I shall have occasion to refer to only two. These both lie in the plane of bilateral symmetry. One, which I shall call the *primary axis,* is the chief axis of the cylinder which the actinian typifies; the other, which I shall call the *secondary axis,* is perpendicular to it.

II. SECONDARY AXIS AND DIRECTION OF LOCOMOTION

In bilateral animals the chief or primary axis so commonly coincides with the direction of locomotion that it is natural to ask whether a like relation exists in the actinians, for, as is well known, many of these animals exhibit bilateral symmetry internally even though their parts seem to be radially disposed externally. The four species studied were all sessile rather than locomotor. Metridium crept rarely and slowly (Fleure and Walton, '07, p. 218, note that Metridium dianthus may move as much as 7 inches in a day), Sagartia moved frequently but still slowly, Condylactis with greater freedom and speed, and Actinia most freely of all.

As a preliminary step specimens of all these species were watched and when creeping was shown by any of them, a record was made of the direction of locomotion in relation to the secondary axis of the animal as indicated by the plane in which the mouth was elongated. Thus a large brown specimen of Metridium, whose pedal disc had a diameter of about 7 cm., was observed to creep up the vertical side of a glass aquarium for a distance of some 10 cm. and during the whole of its course the secondary axis was approximately at right angles to the direction of locomotion. In another instance a specimen crept upwards in a jar over a stretch of about 9 cm. with the secondary axis coincident with the direction of locomotion. In a specimen of Condylactis whose pedal disc measured about 13 cm. by 8 cm., the direction of locomotion also coincided with the secondary axis. In Sagartia a specimen with a pedal disc measuring 15 mm. by 8 mm. was seen to creep over a distance of 4 mm. with this axis in exact agreement with the direction of motion, though in a second specimen the secondary axis was observed to be almost precisely at right angles to the direction of creeping. In six

other individuals the line of locomotion in each case was oblique to this axis.

These preliminary observations made it quite clear that the actinians under consideration not only do not creep invariably in a direction corresponding to their secondary axis, but that their directions of locomotion bear no simple relations to such axes. Is this relation constant for each individual, or does it change from time to time in the same animal? An answer to this question was sought in the two more easily controlled species, Actinia bermudensis and Sagartia luciae. Actinia bermudensis when placed in a large, flat-bottomed, glass dish is very likely to attach itself quickly to the glass surface and begin creeping. The directions of its movements are by no means constant. How diverse they may be can be indicated by the records from a single animal. Designating the direction of the secondary axis in this animal as north and south, the successive creeping movements made by it were found to be as follows: Five movements one after another were first taken in what would correspond to a westerly direction, then one to the northwest, followed by one to the north, after which the animal made three movements to the southwest, then three to the west, and finally two midway between west and southwest. All these movements were carried out without shifting the secondary axis from its original north-south direction.

Still more conclusive results were obtained from Sagartia luciae, which I have had the opportunity of working on since the publication of my preliminary note (Parker, '15). This species is negatively phototropic[2] and the direction of its movements can be controlled easily by changing the direction of the light. A monoglyphic specimen was induced by a beam of strong light to creep in the direction of its secondary axis. The light was then shifted through 90 degrees, and in about 10 minutes the animal had changed its course correspondingly but without turning its body. It was thus induced to creep at right angles to this axis. The light was next set at about 45 degrees to its first

[2] Contrary to the statement made by Hargitt ('07, p. 280) I have found Sagartia luciae very responsive to light.

position and the actinian after a short interval began again to creep away from the light and without changing the direction of its secondary axis. Three other specimens were tested in essentially the same manner and gave similar results. In all cases new courses of locomotion in close agreement with the direction of the light were established in from 10 to 15 minutes after the change in the position of the light was made and the animals could thus be induced to creep at any angle to their secondary axis. In other words, locomotion in actinians is radial in character and is not limited by the structural bilaterality of these forms. This bilaterality, then, is not locomotor, as it is in most of the higher animals, but must be associated with some other general function. What this function is cannot be stated with certainty, but the fact that the bilaterality of the sea-anemones centers around the mouth which, though a single opening, is elongated and differentiated for simultaneous incurrent and excurrent streams, and thus serves respiration, the appropriation of food, and the discharge of excrement, suggests that these rather than locomotion are some of the activities whose influences have brought about the bilateral symmetry of the sea-anemones.

III. MECHANICS OF PEDAL LOCOMOTION

In all the actinians I have studied, locomotion was accomplished by a wave-like movement that passed over the pedal disc. This would begin at the rear edge of that organ and proceed thence to its front edge. Such movements are almost always accompanied by an elongation of the pedal disc in the direction of locomotion as observed by Osburn ('14, p. 1165). In Metridium these waves have been briefly described and figured by McClendon ('06), who ('11, p. 61) has also identified them in Cradactis. So far as I am aware, however, the details of this movement have never been very fully investigated.

In a specimen of Sagartia whose pedal disc had a diameter of about 4 mm. the waves could be seen coursing from the rear to the front as the animal crept over the level bottom of a glass dish. In 15 minutes 5 successive waves had passed across the disc and the animal had crept about 6 mm. The slowest wave

required 2.5 minutes to traverse the disc, the most rapid 1 minute, the average time being 1.65 minutes. The distance progressed as a result of each wave was on the average 1.2 mm. Each wave began by a general contraction of the posterior edge of the pedal disc whereby that portion of the disc was lifted off the substratum and crowded toward the front. This wave of contraction gradually proceeded toward the middle of the disc and the posterior edge was then brought down on the substratum a little in advance of its former position. The wave now reached the anterior edge of the disc and, while the middle was being reattached to the substratum, the anterior edge, now freed, was advanced and finally brought down somewhat in front of its former position. Thus the passage of a single wave carried the whole pedal disc forward for a short distance and as a result the animal changed its location. It is doubtless the front portion of this wave that Torrey ('04, p. 204) speaks of in Sagartia davisi as a multicellular ameboid process by which this species could creep more than an inch an hour.

In Actinia bermudensis the pedal waves were quite like those seen in Sagartia except that they were of more considerable dimensions. In a specimen whose pedal disc measured 30 by 27 mm. the complete passage of a single wave required from 3 to 3.25 minutes and at each wave the animal progressed from 4 to 4.5 mm. In Metridium, whose pedal disc is about the same size as that of Actinia bermudensis, the locomotor wave passes over the disc, according to McClendon ('06), in the short interval of about a minute.

In Condylactis, because of its large size, the waves were more satisfactorily studied than in either Sagartia or Actinia. In a specimen whose pedal disc measured 130 by 80 mm., the waves passed over the disc in from 3 to 4 minutes and for each wave the animal progressed a distance varying from 5 mm. to as much as 17 mm. In another instance 10 waves were seen to pass in 30 minutes and the animal progressed in this interval 114 mm. Thus the average period of each wave was 3 minutes and the average distance advanced for each wave was 11.4 mm. As the wave passed over the pedal disc of Condylactis, it was easy to

see in the region of activity that each part of the disc was successively lifted off the glass, advanced, and put down again. This was suspected to be the case in Sagartia and has been intimated for Metridium by McClendon ('06).

The accompanying diagram (fig. 1) illustrates the steps by which these waves may give rise to actual locomotion. At stage

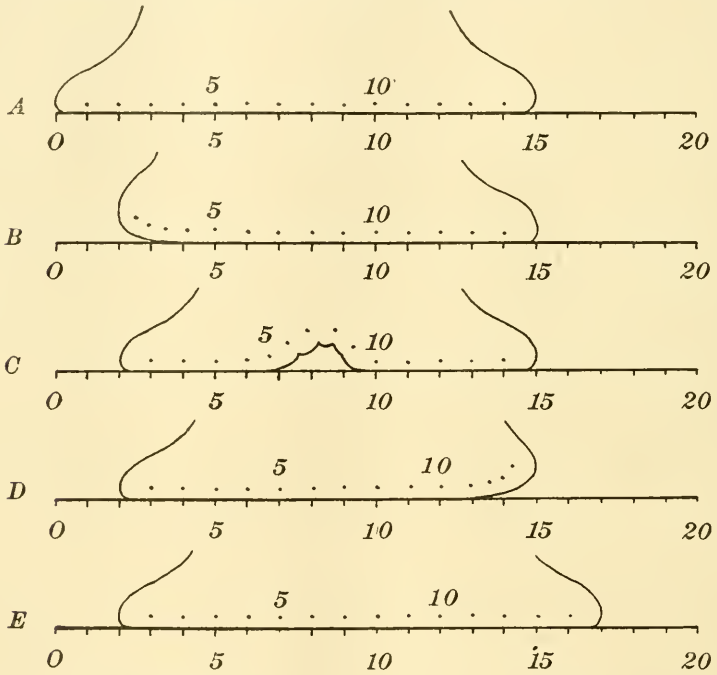

Fig. 1 Diagrammatic side-view of the foot of a creeping actinian illustrating the passage of a locomotor wave from one period of rest (*A*), through a period of activity (*B, C, D*) to a second period of rest (*E*). Fixed points on the substratum are numbered *0, 5, 10, 15,* and *20*. Points in the actinian that at the beginning were opposite the fixed points *5* and *10* in the substratum are numbered also *5* and *10*.

A a pedal disc is supposed to be at rest on the substratum, and corresponding points in the disc and on the substratum are similarly numbered. At *B* the locomotor wave has made its appearance at the posterior edge which has been lifted off the substratum and moved forward. At *C* the wave has reached the middle of the disc and the posterior part has reattached itself in

advance of its former position. At *D* the wave has reached the anterior edge, which is now beginning to be moved forward. At *E* the whole disc is at rest again but in a position in advance of its former situation.

I think there can be no doubt that this type of movement was observed by Gosse ('60, p. 81) in Sagartia pallida though somewhat inaccurately described by him. Gosse states that this species, which crept away from the light and covered as much as thrice its length in a quarter of an hour, accomplished this movement by pushing forward the front part of its pedal disc and attaching this portion, after which the hind part was loosened, drawn up to the front part, and reattached. This description, which agrees with that recently given by Fleure and Walton ('07, p. 218), implies a type of motion like that of a measuring-worm, and is probably a slightly inaccurate account of the wave movement seen by McClendon and me, from which it differs after all only slightly.

In all the creeping actinians that have come under my observation I have never noticed more than a single wave on the pedal disc at one time. In my experience a given wave runs its full course and completely disappears at the front edge of the disc before a second wave begins to form at the hind edge. McClendon ('06), however, states that he has seen two waves on the disc of Metridium at the same time. I know of no reason why this may not occur especially when the direction of creeping is well established.

The direction in which the pedal wave is propagated always agrees with that of the locomotion. The waves on the actinian disc may, therefore, be called direct waves to borrow a term introduced by Vlès ('07) for the waves on the foot of the gastropod. But this is not the only resemblance of the actinian wave to that on the gastropod foot. As the preceding description shows, the two movements are mechanically identical, for in the locomotion of the gastropod, as in that of the actinian, each point on the foot is successively lifted, moved forward, and put down (compare Parker, '11).

In another respect also the creeping of the actinian agrees with that of the gastropod. As Osburn ('14, p. 1165) pointed out

for Sagartia, an actinian as it creeps leaves behind it a trail of slime much as a snail does. This slime is produced by the many unicellular glands in the ectoderm of the pedal disc and undoubtedly serves to increase the adhesion between the disc and the substratum. When the resting place of one of the more sessile actinians, like Metridium, is examined, it is usually found heavily coated with slime, which is often partly solidified into a membrane of a more or less brownish color. In assuming this relatively fixed condition the pedal disc of Metridium gradually spreads out over a comparatively large area and by the secretion of slime the actinian may anchor itself very firmly to the substratum. In this operation the region of most effective attachment is the edge of the disc rather than its center. Nevertheless the disc does not appear to act as a sucker, a fact which is probably correlated with the absence of a parieto-basilar muscle in this species.[3] Thus the pedal disc of Metridium, like the foot of the gastropod, may serve as a holdfast as well as an organ of locomotion (Parker, '11). In this respect, however, the disc must not be looked upon as a fleshy organ that simply adheres to the substratum in a purely mechanical fashion. It has often happened that the large actinians, Metridium and especially Condylactis, when in full locomotion, would meet with difficulty in loosening the active part of their discs from the substratum. Most of the disc would easily become free, but particular points would often continue to adhere to the substratum until after much straining they would suddenly break away. The impression made at the time was that these points of adhesion represented minute well localised organs of attachment, but whether such really exist in addition to the general surface of the disc I can not say.

IV. STRUCTURE OF THE PEDAL DISC

The parts directly concerned with the creeping of actinians are the muscles in the pedal disc and in the immediately adjacent region as well as the fluid contained therein. In Metridium,

[3] In other species of actinians in which there are parieto-basilar muscles the pedal disc may act as a sucker as surmised by Piéron ('06), Hérouard ('11) and others.

as is well known (compare Parker and Titus, '16), all the muscles of the pedal-disc region are entodermic, there being no ectodermic muscles in this part of the animal. Next the supporting lamella of the disc and on its entodermic face is an extensive system of circular muscle fibers, the circular muscle of the pedal disc, covering the whole inner surface of the disc and arranged concentrically with respect to it. To the inner face of the pedal disc are attached in radial arrangement all the mesenteries; the larger ones extending from the outer edge of the disc to its center, the smaller ones reaching from the edge only part way to the center, and the smallest ones being limited almost to the edge itself. Each mesentery carries on its two sides and next its region of attachment to the disc slight muscle-bands, which extend from near the edge of the disc to or at least towards the center of this structure. These muscle bands, which are known as the basilar muscles, have therefore radial courses and cross the circular fibers of the disc at right angles. Beside the circular fibers and the radial fibers just mentioned, the longitudinal fibers of the mesenteries terminate in the disc, many of them passing almost perpendicularly from the disc into the mesenteries. It therefore follows that the pedal disc at most points, but especially towards its edge, may be said to be provided with three systems of fibers mutually at right angles to one another, the whole arrangement being strictly on a radial plan. The supporting lamella of the pedal disc like that of the wall of the column contains a rich supply of interlacing nerve fibers (Parker and Titus, '16).

The creeping movements of the pedal disc are carried out by the neuromuscular mechanism just described. As the locomotor wave begins at the hind edge of the disc, the initial contraction probably includes all three sets of muscles but especially the radial fibers and the mesenteric fibers. These muscles work on parts whose fluid contents are under some pressure from the general muscle tonus of the body as a whole.

As the wave passes over the pedal disc and approaches the front edge of that organ, this edge can be seen to be much distended by the pressure of its fluid contents. The walls of the edge are thin and bulge out between the mesenteries which serve

as internal resisting supports and are marked by depressed lines on the surface. If one of these bulging faces is punctured by a needle, water will flow freely from it and the chamber will collapse for a time. It is thus clear that the projection of the front edge of the disc as the locomotor wave reaches it, is dependent upon the pressure of the contained fluid acting on the relaxed walls of that region. How considerable this pressure is has not been determined, but it is without doubt the chief factor in forcing the anterior edge of the disc forward. A small glass tube carefully inserted into the body of a large creeping Metridium showed an internal pressure in one instance of 5.3 cm. of water and in another of 6 cm. Both these pressures must be decidedly above the normal, for in both instances the insertion of the tube caused the animals to contract somewhat and thereby to increase their internal pressure. The mechanism of creeping, then, depends upon the action of the muscles of the pedal disc and its immediate vicinity on the fluid which is contained in the animal under a pressure of less than 5 or 6 cm. of water. This method of producing locomotion, without the participation of skeletal parts, has long been recognized in many invertebrates and is generally admitted for such organs as the foot of the gastropod.

Ever since the work of the Hertwigs ('79–80) it has been customary to assume that the normal neuromuscular activities of the actinian body were more or less under the control of at least slightly centralized nervous organs, which were believed to be located in the oral disc. To what extent are the creeping movements of the pedal disc dependent upon such oral centers? To answer this question experiments were carried out on Sagartia luciae. Fully expanded, attached specimens of this actinian were suddenly cut transversely in two with a pair of sharp scissors. The oral pieces thus cut off and carrying with them in each case the whole of the oral disc, tentacles, etc., were discarded. The attached pedal discs and remaining portions of the columns were held under careful observation. These contracted at the level at which they had been cut so as to look like an actinian normally withdrawn. They soon filled them-

selves with water and in 12 to 15 hours after the operation many of them were creeping about precisely as the whole animals did. A single record will illustrate this. One of the animals without its oral disc began creeping and was observed to carry out 4 movements in 16.5 minutes traveling in that period 7 mm. An animal with its oral disc intact that had been kept under similar conditions as a control carried out 4 locomotor movements in 22.5 minutes, travelling in that period 6 mm. As the differences between these two sets of records are no more than may be seen in any pair of normal individuals, the locomotion of the two animals may be regarded as essentially identical. Individuals without oral discs not only creep as whole individuals do, but they also attach themselves to a glass surface as firmly as do those with oral discs. Furthermore those without oral discs creep away from the light as consistently as do normal individuals. In fact, so far as the creeping is concerned, it is impossible to distinguish one class from the other except perhaps that the operated animals are somewhat less inclined to creep than the normal ones are.

The conclusion to be drawn from this experiment is that the pedal disc and its immediately adjacent parts contain all the neuromuscular mechanism that is necessary to creeping, in other words, this function is in no sense dependent upon the assumed nervous centers of the oral disc. These results are in entire accord with Loeb's investigations ('95, p. 418; '99, p. 34) in which he has shown that an Actinia equina from which the oral end has been cut off will creep more or less continuously on glass, and will attach itself firmly to a mussel shell just as a normal animal will. They are also in accord with Jordan's results ('08, '12) in which he has shown that the reflex excitability and muscle tonus of actinians is not under the control of superior nervous centers. They are quite at variance with such views as those expressed by Gosse ('60, p. 81), who in speaking of the creeping of Sagartia pallida states that "it was impossible to witness the methodical regularity of the process and the fitness of the mode for attaining the end, without being assured of the existence of both consciousness and will in this low animal form." In this respect the

pedal disc is like an actinian tentacle which on being cut off will, if properly handled, carry out temporarily all the reflexes that it exhibited while it was a part of the animal as a whole.

The creeping of an actinian is by no means a simple process. Not only is a rather complex wave of locomotion made to pass over the pedal disc from one side to the other, but this wave may originate at any point on the edge of the disc and pass through its center to the opposite edge; in other words, the disc appears to possess potentially an infinite number of axes of locomotion. From this standpoint its strictly radial structure conforms well with its activities, for a system of circular and of radial fibers in a horizontal plane combined with a set of approximately vertical fibers gives just that structural combination necessary for unrestricted radial locomotion. The effective symmetry of the actinian pedal disc is, then, a locomotor symmetry for it is strictly radial; the symmetry of the oral disc, as already pointed out, is bilateral and is in no direct way connected with locomotion.

What it is that induces the formation of locomotor waves on any particular side of the pedal disc of an actinian, is not easy to discover. Probably many stimuli are effective in this respect. The fact that Sagartia and many other actinians creep away from light suggests that the light itself may stimulate the mechanism concerned with the formation of the locomotor waves, for in negative animals such as these the light falls most strongly on that face of the animal where the waves originate.

Sagartia likewise commonly creeps up the sides of glass jars and it will do this even when the top of the jar is closed and contains no air. Such a reaction would, therefore, seem to be of a geotropic kind and dependent perhaps upon the deforming pressure exerted on the lower edge of the pedal disc of this actinian when attached to a vertical support, for the body of Sagartia is slightly heavier than sea water and would tend to sag downward from a horizontal position. However this may be, some stimulus must be present to start from a given side the locomotor wave which then courses in a determined direction across the pedal disc to be followed often by others whose presence calls

apparently for no more mechanism than is found in the relatively simple neuromuscular structure of this organ acting on the fluid retained under slight pressure in the adjacent body spaces.

V. SUMMARY

1. The direction of creeping of actinians is independent of their secondary axis. In a single specimen of Actinia or of Sagartia the direction of creeping may change from time to time without relation to the secondary axis. The bilaterality of actinians, therefore, is not locomotor as in most animals. It is probably respiratory.

2. Actinian locomotion is accomplished by a wave-like movement which progresses over the pedal disc in the direction of locomotion.

3. In a specimen of Sagartia with a pedal disc about 4 mm. in diameter, the locomotor wave coursed over its disc in an average time of 1.65 minutes and with each wave the animal progressed on the average 1.2 mm. In a specimen of Condylactis with a pedal disc 130 by 80 mm., the passage of a locomotor wave required on the average 3 minutes and the animal progressed for each wave on the average 11.4 mm.

4. In the actinian locomotor wave each point on the pedal disc is successively raised from the substratum, moved forward, and put down.

5. The attachment of the actinians studied to the substratum is due chiefly to adhesion heightened by the secretion of a thick slime rather than to a sucker-like action of the pedal disc.

6. The mechanism of locomotion consists of the circular muscle of the pedal disc, the basilar muscles, and the longitudinal muscles of the mesenteries, all of which act on the fluid-filled spaces in the pedal region of the actinian. The pressure thus generated is not above that of 6 cm. of water.

7. Creeping can be accomplished by an actinian from which the oral disc has been cut away. Hence the pedal portion of actinians, like their tentacles, contains a neuromuscular mechanism sufficient for its own activity.

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THE EXPERIMENTAL MODIFICATION OF GERM CELLS

I. GENERAL PLAN OF EXPERIMENTS WITH ETHYL ALCOHOL AND CERTAIN RELATED SUBSTANCES[1]

RAYMOND PEARL

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THREE FIGURES

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Although every variation is either directly or indirectly caused by some change in the surrounding conditions, we must never forget that the nature of the organization which is acted on essentially governs the result.—CHARLES DARWIN.

I. THE PROBLEM

One of the outstanding problems of genetics is that of the origin of new heritable variations. With the passage of time and the accumulation of exact experimental data it becomes increasingly clear that this factor is the basic one in all evolutionary change, whether progressive or retrogressive. Just now it is the fashion to speak of new heritable variations as mutations, but such designation does not appear either to make the facts concerned any different from what they were under

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 100.

an older terminology, nor does it essentially contribute to our knowledge about them. Indeed it is, so far as I can see, entirely fair to say that but little in the way of essential advance has been made towards the solution of this problem since Darwin's examination and analysis of it. The two leading students of variation since Darwin (Bateson and De Vries) have, to be sure, contributed greatly to our knowledge of certain aspects of the phenomena of variation; notably, on the one hand, in the direction of establishing a number of definite principles or laws of morphogenesis which control or determine in large degree the somatic expression of germinal differences, and, on the other hand, in very precisely and minutely analyzing the genetic behavior of various heritable variations, after they have appeared. But it is the problem of the origin, the determination, the causes of those germinal differences which lie behind somatic variations, and indeed are the heritable variations, which appears to be the basic problem of genetics (Pearl, 21, p. 39).

One possible method of attacking the problem of the origin of heritable variations is that suggested by the quotation which stands at the beginning of this paper. One may systematically expose the germ-cells of an animal to something unusual or abnormal "in the surrounding conditions," and then analyze, so far as may be, not only the new heritable variations themselves (provided any such appear), but also the factors which underlie their causation. One is the more encouraged to undertake experimentation in this direction, because of the very interesting results of such studies which have been reported during the last few years, particularly those of Stockard (34, 35, 36, 37, 38), and Cole and his students (3, 4), with mammals and birds. In this connection mention should also be made of the work of Sumner (39) with mice, Kammerer (13) with lower vertebrates, Tower (41) with insects, and MacDougal (15) with plants. No attempt will be made at this point to review this literature. Such observations of these others workers as relate to the results here reported will be discussed later in this series of papers.

In discussing with Professor Stockard in 1914 his very interesting results on the effect of chronic parental alcoholism upon

the progeny I suggested that it seemed highly desirable that experiments along similar lines should be carried out with some bird as material. The differences in mode of reproduction between mammals and birds offered an opportunity, as it seemed to me, to check certain possibly doubtful points in the interpretation of the guinea-pig results. Upon Professor Stockard's very kind assurances that he should regard this as in no way an encroachment upon his field of work, I started the experiments here reported in the early autumn of 1914.

The problem of the effect of parental alcoholism upon the progeny was first assigned to Mr. H. R. Barrows as a subject for a doctorial thesis. The actual management of the experiment and the taking of the routine data were in the hands of Mr. Barrows from September, 1914, to about March, 1915. At about that time, for reasons which need not here be gone into, the problem and the experiments reverted to the writer. For efficient and timely aid in various phases of the conducting of the experiment, collecting the quantitative data, and computing the results I am greatly indebted to the following assistants in this laboratory: Dr. Maynie R. Curtis, Mr. John Rice Miner and Mr. H. T. Covell, as well as to Mr. H. R. Barrows, as already mentioned.

The problems with which this investigation deals are specifically these:

1. Does the continued administration of ethyl alcohol (or similar narcotic poisons) to the domestic fowl induce precise and specific changes in the germinal material, such as to lead to new, heritable, somatic variations?

2. Failing a specific effect, is there a general effect upon the germinal material leading to general degeneracy of the progeny?

3. What in general are the effects upon the soma of the treated individual of the continued administration of such poisons?

4. Are the somatic effects upon the treated individuals of a sort to give any clue as to the probable origin, or mechanism of the germinal changes?

The general plan adopted for the presentation of the results of these alcohol experiments is as follows. In this first paper

in the series the general methods used in the conduct of the experiments will be described in detail. Such a thorough and detailed description of the stock used in the work, the experimental methods, etc., is believed to be essential if the reader is to form any adequate idea of the critical value of the results obtained. The writer is very anxious that any one who reads the papers in this series shall have the opportunity of knowing exactly how the work was done. If then he desires to controvert the conclusions reached he will at least be saved the trouble of interpreting the results on the basis of totally erroneous suppositions as to the technique of the work, the stock used, etc.

The second paper in the series will contain an account of the effect of the treatment with alcohol upon the treated individuals themselves.

The third paper in the series will set forth the results obtained regarding the effect of the treatment of the parents upon the progeny.

Inasmuch as these three papers will appear in succession in the same journal it has seemed desirable, in the interest of economy of space, to print the numbered list of bibliographical titles cited only once and in connection with this, the first paper in the series. The numbers following citations in the next two papers in the series will, therefore, be understood to refer to the bibliography appended to the present paper.

II. MATERIAL AND METHODS

A. General Plan

The general plan of this investigation involves some features which have not been incorporated in earlier researches in this general field. In the first place, it was thought desirable to use two pure breeds of poultry for the foundation stock in the experiments rather than one, and in consequence of this make the offspring of the treated animals F_1 crossbreds rather than pure-bred birds. The primary consideration in favor of this plan was that, by its adoption, a much more manifold opportunity seemed likely to be given to test any putative influence

of the poisons on the germ plasm. It should be possible in an experiment of this sort to see whether in F_1 the usual conditions as to Mendelian dominance are in any manner or degree disturbed by the administration of the poisons to the parents. Further, when the F_1 individuals from treated parents are themselves bred there will be an opportunity to apply the most delicate of all genetic tests for the composition of the germ plasm, namely the test of segregation in F_2 and succeeding generations.

A further possible advantage of the plan of having the treated individuals belong to two distinct pure breeds, or races, arises from the fact that advantage will then be taken of any increase in physiological vigor, if any such occur, which arises from the condition of heterozygosis (East and Hayes, 6). In any breeding experiments of this sort it is highly desirable, for purely practical reasons, to start at least with as high a degree of general constitutional vigor in the animals as is possible, because it will make the getting of progeny in large numbers by so much the easier.

There appears to the writer to be no disadvantages of any weight which can be brought forward against the plan of using two pure breeds and making the progeny cross-bred animals in such an investigation as this, provided one is thoroughly acquainted with the strains which he uses to start the experiments. In the investigation here reported the foundation stock used came from pedigreed strains of two breeds, Black Hamburgs and Barred Plymouth Rocks. Both of the strains used have been so long pedigree-bred by the writer, and used in such a variety of Mendelian experiments, that they may be regarded as reagent strains, whose genetic behavior under ordinary circumstances may be predicted with a degree of probability amounting practically to complete certainty. Furthermore the results of crossing these two breeds reciprocally have been thoroughly studied by the writer. The large amount of material which has accumulated in this laboratory, showing the genetic behavior in a Mendelian sense of these two particular strains when crossed together under normal circumstances may, to a

large extent, be used as a control on the alcohol experiments with the same forms.

To those not entirely familiar with the breeds of poultry it may be helpful to call attention to certain of the more important characteristics of these two breeds. To this end figures 1 and 2, and table 1 are presented. Figure 1 shows a normal Black Hamburg male and figure 2 a normal Barred Plymouth Rock female. These are the forms used in these particular experiments (p. 138).

It is not the intention to give any detailed account in the present paper of the mode of inheritance of the differences between the two breeds exhibited in table 1. Full discussion of these matters is left for consideration in another paper, in which normal, untreated birds only will be dealt with. In the third paper of this series certain details that are necessary to the understanding of the alcohol experiments will be given regarding the normal inheritance of these characters.

TABLE 1

Showing the chief characteristics of the Black Hamburg and Barred Plymouth Rock breeds of poultry

CHARACTER	BARRED PLYMOUTH ROCKS	BLACK HAMBURG
Plumage color	Black and white	Black, with greenish sheen
Plumage pattern	Barred	Self
Comb	Single	Rose
Ear lobes	Red	White
Shank color	Yellow	Black or leaden blue
Plantar surface of feet	Yellow	White
Beak	Yellow	Black or dark horn
Skin color	Yellow	White
Egg color	Brown	White
Body size (standard weights)[2]	Large: cockerel = 8 lbs. pullet = 6 lbs. cock = 9½ lbs. hen = 7½ lbs.	Small: There are no "standard" weights. Pullets average about 2½ lbs. and hens about 2¾ to 3 lbs. Male birds 3½ to 4½ lbs.
Egg-laying ability	Good (cf. Pearl, 20)	Poor

[2] Cf. American Standard of Perfection, 1915.

Fig. 1 Normal Black Hamburg male

Fig. 2 Normal Barred Plymouth Rock female

B. Substances used and mode of administration

In the present investigation three different series of birds were started. To the birds in one series was administered 95 per cent ethyl alcohol (C_2H_5OH). To those in the second series was administered methyl alcohol (CH_3OH) and to those in the third series ether $(C_2H_5)_2O$. In all cases, on account of the expense involved, use has been made of standard commercial preparations of these three substances, rather than the most refined, chemically pure, reagents. It is entirely unlikely that in such an experiment as this any difference could by any possibility result from the use of standard commercial substances rather than strictly chemically pure reagents. Furthermore in regard to this, as well as to some other similar points which may occur to one in reading these papers, it will be well to remember that after all the primary object of the experimentation was genetic, not pharmacological. In other words, the purpose of the investigation is to produce, if possible, some change in the germ plasm of the treated individuals. It will be time to undertake the minute analysis of just what the chemical nature and cause of the change is, after the change itself has been produced. For beginning experiments, such as those here reported, too extreme refinement of method at unessential points contributes nothing to the research and may tend actually to hinder rather than help the getting of any results at all.

The method followed in these experiments for the administration of the poisons was essentially that which has been used by Stockard, namely the method of inhalation. Some years ago the present writer carried out an experiment with Mr. B. A. Ahrens, then a student of the University of Maine, in which the attempt was made to administer ethyl alcohol regularly to a flock of birds in the drinking water. It was found impossible to use this method with any degree of satisfaction for precisely the same reasons which Stockard and Papanicolaou (38, p. 69) have found to militate against its use with guinea-pigs. Fowls will not drink water containing even dilute solutions of ethyl alcohol if they can possibly find any other way of reliev-

ing the pangs of thirst. As a matter of fact, the fluid consumption of the birds so treated was very greatly reduced below that of the untreated controls. On the other hand, the inhalation method gives very satisfactory results with poultry, just as it has with guinea-pigs.

In the present experiments inhalation tanks of two different sizes have been used. The general form and appearance of one of these tanks is shown in text figure 3.

The general plan of construction of these tanks will be evident from figure 3. They are essentially square boxes of galvanized iron, having at the top a round opening which serves as a means of entrance and exit for the bird. This opening is tightly closed by a cover during an experimental treatment. Below the bottom of the tank is a cylindrical reagent chamber closed by a tight fitting cover from below. In this projection below the floor of the tank proper is placed absorbent cotton saturated with the particular reagent used. Over the top of the reagent chamber is placed a piece of heavy galvanized wire gauze of about half-inch mesh which serves to complete the floor of the inhalation compartment proper, without obstructing the diffusion of the fumes from the reagent chamber.

The dimensions of the tanks used are as follows: Large tank: length 2 feet, width 2 feet, height 21 inches, top cover hole 15 inches in diameter. The reagent chamber at the bottom is 8 inches in diameter and 6 inches deep. The window in front is $15\frac{1}{4}$ by 9 inches. These large tanks have a capacity of four or five birds at one time. The volume of contained air in one of these large tanks amounts to something over 7 cubic feet.

The small tanks have the same dimensions as the large except in the following particulars: length 18 inches, width 18 inches, window in front 12 by 9 inches. These smaller tanks will accommodate two or three birds. The volume of contained air in one of these small tanks is about 4 cubic feet.

No special arrangements have been found necessary in these tanks for ventilation. The number of birds treated in any one tank at a time is kept down to the point where they could exist for a long time without any discomfort owing to exhaustion of

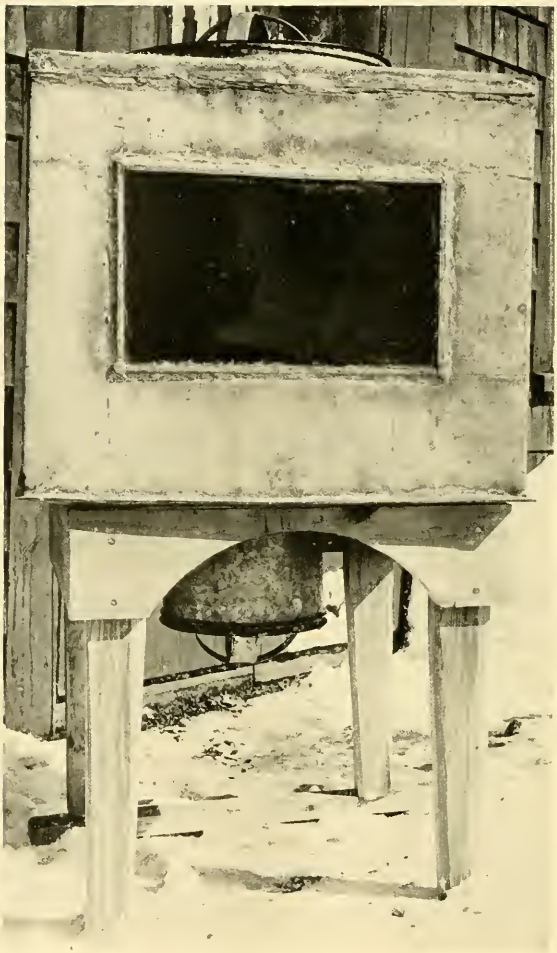


Fig. 3 Showing one of the inhalation tanks used in the administration of poisons in this experiment. For description and dimensions see text.

the air. It has been found by experiment that a single bird will remain in one of the large tanks above described, tightly closed so that no air from the outside can get in, for at least 12 hours without showing any signs whatever of discomfort, and probably would stay much longer than that. Trial has shown that even as many as five large birds may be put in one of the

large tanks and, in the absence of any reagent, will show no signs of discomfort from lack of air for a period of at least three hours.

Regarding the mode of administration of the poisons used it was found early in the work to be undesirable to depend entirely upon the evaporation of the reagent from cotton in the chamber at the bottom of the tank. This process took altogether too long a time to saturate the air of the tank with the vapor. Practically from the beginning we have used a combination of this method plus a preliminary saturating of the air with the vapor of the substance used by means of an atomizer. The routine procedure is this: there is placed in the reagent chamber at the bottom of the tank a piece of absorbent cotton soaked with the reagent to be used, ethyl or methyl alcohol or ether, as the case may be. Then the operator quickly but thoroughly fills the whole of the tank proper by means of an atomizer with a saturated vapor of the same substance. The birds to be treated are then introduced quickly, allowing as little as possible of the vapor to escape in the process. When the birds have been introduced the cover of the tank is tightly closed and left in that condition for one hour. It is to be understood throughout this paper that every bird designated as a 'treated bird' has spent one hour every day in one of these tanks subjected to the fumes of the reagent specified in the particular case. At the beginning of the experiments it was thought desirable to accustom the birds gradually to the vapor treatment, and consequently in some cases the treatments for the first week were only one-half hour in duration. It was soon found, however, that a sound healthy bird could stand the treatment for an hour, even from the first. Consequently in all later work it has been the rule to make the treatment extend over one hour each day from the very beginning.

It is impossible, as Stockard has pointed out, to make any very precise statements regarding dosage when a reagent is used by the inhalation method. The time of subjection to the fumes is perhaps the most significant measure that one can get. It may not be without interest, however, to note the amounts

of the reagent actually evaporated into the tank during each day's treatment. These amounts in the experiments here reported are for the large tanks about 45 cc. per treatment, and for the small tank about 30 cc. per treatment. Taking into account all the factors in the case it appears reasonable to consider that the degree of alcoholization in these birds corresponds fairly to that of the steady but moderate drinker.

In a later paper in this series data on the immediate physiological effect on respiration rate, temperature, etc., of the administration of alcohol by the inhalation method will be presented. From these data it is possible, by the same sort of reasoning as is used in the so-called physiological assay of drugs, to arrive at a fair idea of the effective physiological dosage when substances are administered by inhalation.

Regarding the time factor here used it should be said that one hour is approaching closely to the limit of time which the birds will survive the fumes. This is proven by the fact that in the course of the experiments nearly all of the birds in two series have been lost in two accidents. In both cases the birds were left for a short period beyond the alloted time of one hour, and were promptly killed beyond possibility of reviving. The first of these accidents took place on April 15, 1915, when the attendant forgot that the birds were in the ether tank and allowed them to remain in there for about an hour and twenty minutes. The four birds, 1490, 1391, 1572 and 1573, which were in the tank at that time were found to be past any hope of revival by first aid methods, which were vigorously applied. Male No. 665 was in the same tank with the females mentioned and was nearly dead, but subsequently revived and lived for nearly another year after, until he was killed for experimental purposes. Fortunately one ether female, 1585, was not in the tank at the time of this accident. On September 5, 1915, a similar accident resulted in the death of three methyl birds, 1486, 1487 and 1492. The conditions of the accident were much the same as those already described in the case of the ether birds. At this point in the paper, the important feature regarding these accidents lies in the fact that they demonstrate that the dosage

used was practically the maximum possible under the conditions of administration here employed.

Regarding the immediate effect of the inhalation treatment of birds with these poisons it may be said that in general our experience with fowls is quite similar to that of Stockard with guinea-pigs. As a result of the daily handling, the treated birds become very tame and learn their way from the tanks to their pens so that when the tanks are opened at the end of the treatment the birds go without any attention from anyone back to the pen in which they live. Occasionally one of the birds gets staggery at the end of the treatment, but otherwise there is little in the behavior or appearance of these birds to indicate that they have undergone daily treatment for more than seventeen months. The effect of the vapor on the eyes of the birds is somewhat similar, though not so marked in degree, as that described by Stockard and Papanicolaou (38, p. 72) for guinea-pigs. None of the hens has become totally blind, though the immediate effect of the vapor in producing a milky secretion is usually though not always, observed. It seems surprising that no more pronounced toxic effects from the methyl alcohol have appeared. The only explanation which I can suggest for this is that since the individual daily doses are non-toxic an acclimatization has resulted in the course of continued treatment, so that the surviving birds are very resistant to methyl alcohol poisoning. It is worthy of note in this connection that it is the two more directly toxic substances used, methyl alcohol and ether, with respect to which our two tank accidents have happened, resulting in the death of the birds. More extended and precise data on the effects of the substances on the general physiological economy of the treated birds will be given in the second paper in the series.

In regard to the general environmental conditions under which the experiments were carried out it may be said that these birds were fed, housed and managed according to methods which long experience in poultry work at this Station has shown to be excellently adapted to the preservation of the health and the maintenance of the physiological vigor of poul-

try (Pearl 22). Special pens have been devoted to this experiment from the beginning and the birds have not been moved about from them except for short periods for purposes of cleaning. It is scarcely necessary to add that control and treated birds have been fed and managed in the same way, every effort being made to see that the only differential factor in the management of the two sets of birds was the inhalation treatment.

C. Foundation stock used in the experiments

In table 2 is given a complete conspectus of the foundation stock used.

From this table it will be seen, in the first place, that the birds of the Barred Plymouth Rock breed used were all females, and the birds of the Black Hamburg race used were males. The cross was made in this way since the barred color pattern of the Barred Rock is known to be a sex linked character for which the female is heterozygous (Goodale, 8, Pearl and Surface 28 and others). By making the cross in this way additional possibilities in the variety of character combination are opened. In the second place, it is evident from table 2 that the birds were so chosen that each treated bird had from one to five or six untreated sisters to serve as controls. This is believed to be an important critical element in the conduct of the experiments.

Another reason for choosing Barred Rock females for the experiments is that they are a docile breed, easily tamed and not nervous or easily frightened when properly handled. These are important factors in the critical conduct of an experiment of this sort. It is easily within the bounds of possibility, in fact experience would lead one to regard it as even probable, that quite erroneous conclusions might be reached in experimental work of this sort if females of some very nervous, easily excited and flighty breed, as the White Leghorn for example, were used. Daily tanking might very well put then off their laying to a marked degree, even though the tank contained nothing whatever but just plain air. Meantime the untanked controls would be laying normally, and one would be almost sure

to draw the really quite erroneous conclusion that the alcohol caused the poor laying.

Summarizing the data in table 2 it appears that there were six females and one male treated with ethyl alcohol; four females and one male treated with methyl alcohol; and five females and one male treated with ether. Of the females treated with ethyl alcohol, one (1574) was approximately $1\frac{1}{2}$ years old at the beginning of the treatment. The other five (1481, 1482, 1483, 1484 and 1889) were from seven to ten months old at the beginning of the treatment. In the case of the methyl-alcohol birds, one (1575) was a year and a half old hen, and the other three (1486, 1487 and 1492) were pullets. In the case of the other birds, two (1572 and 1573) were hens a year and a half old and the other three (1485, 1490 and 1491) were pullets. All of the male birds used were cockerels from seven to eight months old at the beginning of the treatment. In reading table 2 it should be noticed that the first column headed 'Ex mating' denotes the number of the mating from which the birds in the following column came (Pearl and Surface, 26). The last column headed 'In mating No.' gives the numbers of the matings into which the various birds entered. Of course all of the birds out of the same mating (i.e., having the same mating number in the first column) will be full sisters.

In examining the results of such an investigation as the present one with the purpose of reaching a critical conclusion as to their meaning there is probably no one piece of information which is more earnestly desired by the experienced geneticist than a clear and comprehensive statement as to just how the foundation stock which went into the experiment was bred. This information is commonly denied the reader, not because of any malicious intent to suppress data, but simply because the experimenter himself lacks this knowledge. The inexperienced worker in genetics, and the student of other fields of biology, are not likely to realize the very great importance of the point here involved in reaching a critical interpretation of results. 'Controls' in the ordinary sense may be totally misleading in a genetic experiment, as for example, when the experimented

TABLE 2

Showing the foundation stock used in the experiment

EX MATING NUMBER	BIRD NUMBER	BREED	SEX	HATCHED	DIED	TREATMENT	IN MATING NUMBER
1737	1481	BPR	♀	April 28, 1914	Living Feb. 1, 1916	Ethyl alcohol	2112 (× ethyl ♂)
	1483	BPR	♀	April 28, 1914	Living Feb. 1, 1916	Ethyl alcohol	2127 (× untreated ♂)
	1726	BPR	♀	April 28, 1914	Living Feb. 1, 1916	Untreated	
	M364	BPR	♀	April 2, 1914	Sold Sept. 1, 1915	Untreated	
	487	BPR	♀	April 2, 1914	June 25, 1915	Untreated	2116 (× ethyl ♂)
1785	1482	BPR	♀	April 27, 1914	Living Feb. 1, 1916	Ethyl alcohol	2113 (× ethyl ♂)
	1741	BPR	♀	April 27, 1914	Living Feb. 1, 1916	Untreated	2117 (× ethyl ♂)
	372	BPR	♀	April 21, 1914	Oct. 26, 1914	Untreated	
1788	1484	BPR	♀	April 27, 1914	Living Feb. 1, 1916	Ethyl alcohol	2128 (× untreated ♂)
	69	BPR	♀	April 1, 1914	Feb. 28, 1915	Untreated	Died too soon to be mated
1805	1489	BPR	♀	May 26, 1914	Living Feb. 1, 1916	Ethyl alcohol	2115 (× ethyl ♂)
	331	BPR	♀	April 14, 1914	Sold, Sept. 2, 1915	Untreated	
	346	BPR	♀	April 14, 1914	Sold, Sept. 2, 1915	Untreated	
	1738	BPR	♀	April 27, 1914	Living Feb. 1, 1916	Untreated	2118 (× ethyl ♂)
	296	BPR	♀	April 27, 1914	Sold, Sept. 1, 1915	Untreated	
	292	BPR	♀	April 27, 1914	Sold, Sept. 1, 1915	Untreated	
1568	1574	BPR	♀	April 9, 1913	Living Feb. 1, 1916	Ethyl alcohol	2114 (× ethyl ♂)
	1508	BPR	♀	April 9, 1913	June 5, 1915	Untreated	1760, 1983
	K41	BPR	♀	April 9, 1913	Sold, Aug. 24, 1914	Untreated	
	42	BPR	♀	April 9, 1913	Feb. 8, 1915	Untreated	1761
	45	BPR	♀	April 9, 1913	Sold, Aug. 26, 1914	Untreated	1854
	327	BPR	♀	May 2, 1913	Sept. 9, 1914	Untreated	1801

1790...	1486	BPR	♀	April 27, 1914	Sept. 5, 1915*	Methyl alcohol	2120 (× methyl ♂)
	M36	BPR	♀	April 14, 1914	March 29, 1915	Untreated	1989
	348	BPR	♀	April 27, 1914	Sold, Sept. 1, 1915	Untreated	
	1737	BPR	♀	April 27, 1914	Jan. 22, 1916	Untreated	2123 (× methyl ♂)
1789...	1487	BPR	♀	April 27, 1914	Sept. 5, 1915*	Methyl alcohol	2121 (× methyl ♂)
	1742	BPR	♀	April 27, 1914	Living Feb. 1, 1916	Untreated	2124 (× methyl ♂)
	65	BPR	♀	April 14, 1914	Sold, Sept. 2, 1915	Untreated	
	1683	BPR	♀	April 14, 1914	Living, Feb. 1, 1916	Untreated	1986
	370	BPR	♀	May 4, 1914	Sold, Sept. 1, 1915	Untreated	
	506	BPR	♀	May 4, 1914	Feb. 24, 1915	Untreated	2101
1772...	1492	BPR	♀	April 21, 1914	Sept. 5, 1915*	Methyl alcohol	2129 (× untreated ♂)
	49	BPR	♀	March 31, 1914	Sold, Sept. 2, 1915	Untreated	
	519	BPR	♀	May 25, 1914	Sold, Sept. 2, 1915	Untreated	
	1744	BPR	♀	May 25, 1914	Feb. 6, 1916	Untreated	2131
1536...	1575	BPR	♀	April 7, 1913	Living, Feb. 1, 1916	Methyl alcohol	2122 (× methyl ♂)
	K89	BPR	♀	April 7, 1913	Sold, Aug. 24, 1914	Untreated	
	94	BPR	♀	April 7, 1913	Sold, Aug. 24, 1914	Untreated	
	109	BPR	♀	April 7, 1913	Sold, Aug. 24, 1914	Untreated	
	115	BPR	♀	April 7, 1913	Sold, Aug. 24, 1914	Untreated	
	121	BPR	♀	April 7, 1913	Sold, Aug. 24, 1914	Untreated	
	355	BPR	♀	April 22, 1913	Sold, Aug. 24, 1914	Untreated	
	356	BPR	♀	April 7, 1913	Jan. 26, 1914	Untreated	
	372	BPR	♀	May 21, 1913	Sold, Aug. 24, 1914	Untreated	
	483	BPR	♀	May 21, 1913	Sold, Aug. 24, 1914	Untreated	

* Killed by accident; cf. p. 136.

TABLE 2—Continued

EX MATING NUMBER	BIRD NUMBER	BREED	SEX	HATCHED	DIED	TREATMENT	IN MATING NUMBER
1738...	1485	BPR	♀	April 28, 1914	Living, Feb. 1, 1916	Ether	2126 (× untreated ♂)
	M24	BPR	♀	April 21, 1914	April 8, 1915	Untreated	
	1736	BPR	♀	April 21, 1914	Feb. 6, 1916	Untreated	2131 (× normal ♂)
	118	BPR	♀	April 21, 1914	Dec. 2, 1914	Untreated	
	440	BPR	♀	April 28, 1914	Apr. 21, 1915	Untreated	
1784...	1490	BPR	♀	April 27, 1914	Apr. 15, 1915**	Ether	2105 (× ether ♂)
	1743	BPR	♀	April 21, 1914	Living, Feb. 1, 1916	Untreated	2109 (× ether ♂)
	359	BPR	♀	April 21, 1914	Sold, Sept. 2, 1915	Untreated	
	365	BPR	♀	April 21, 1914	Nov. 2, 1914	Untreated	
1771...	1491	BPR	♀	April 21, 1914	Apr. 15, 1915**	Ether	2106 (× ether ♂)
	1724	BPR	♀	April 1, 1914	Jan. 8, 1916	Untreated	1958 (× BPR ♂)
	60	BPR	♀	April 1, 1914	Sold, Sept. 1, 1915	Untreated	
	55	BPR	♀	April 14, 1914	Sold, Sept. 1, 1915	Untreated	
	1671	BPR	♀	April 14, 1914	Jan. 3, 1916	Untreated	
	1740	BPR	♀	April 21, 1914	Living, Feb. 1, 1916	Untreated	2110 (× ether ♂)
	516	BPR	♀	May 12, 1914	Sold, Sept. 2, 1915	Untreated	
1507...	1572	BPR	♀	April 23, 1913	Apr. 15, 1915**	Ether	2108 (× ether ♂)
	K64	BPR	♀	May 2, 1913	Apr. 17, 1914	Untreated	1868
1575...	1573	BPR	♀	April 10, 1913	Apr. 15, 1915**	Ether	2107 (× ether ♂)
	1511	BPR	♀	April 10, 1913	Sold, Sept. 2, 1915	Untreated	1839, 2054
	1540	BPR	♀	April 22, 1913	Living, Feb. 1, 1916	Untreated	1869
	1549	BPR	♀	April 22, 1913	June 16, 1915	Untreated	1788

1903...	666	BH	♂	April 21, 1914	Living, Feb. 1, 1916	Untreated	2126, 2127, 2128, 2129, 2130, 2131, 2132, 2133
	M241	BH	♀	April, 21, 1914	Feb. 26, 1915	Untreated	
	3257	BH	♂	June 16, 1914	Sept. 9, 1915	Ethyl alcohol	
1901...	664	BH	♂	June 16, 1914	Living, Feb. 1, 1916	Ethyl alcohol	2112, 2113, 2114, 2115, 2116, 2117, 2118, 2119
	463	BH	♀	May 26, 1914	Sept. 1, 1915	Untreated	2009
	469	BH	♀	May 26, 1914	Lost	Untreated	
1900...	663	BH	♂	June 8, 1914	Living, Feb. 1, 1916	Methyl alcohol	2120, 2121, 2122, 2123, 2124, 2125
1896...	665	BH	♂	May 26, 1914	Living, Feb. 1, 1916	Ether	2104, 2105, 2106, 2107, 2108, 2109, 2110, 2111
	1626	BH	♀	May 26, 1914	Living, Feb. 1, 1916	Untreated	

* * Killed by accident; cf. p. 136.

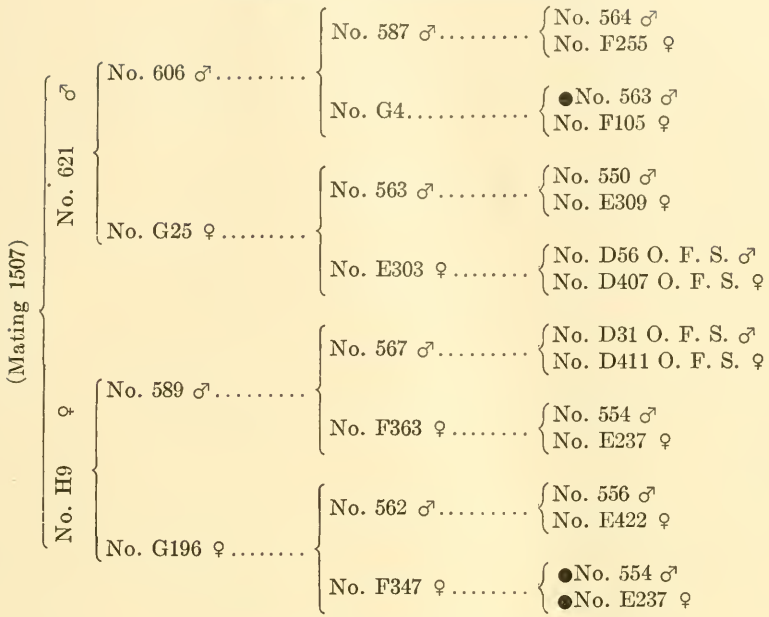
animals and the controls belong to two different strains or blood-lines.

Because of the writer's belief in the fundamental importance of the general problem with which this paper has to do it seems desirable to take the space necessary to give complete data as to the breeding of all the foundation animals used in this study, covering a period of four years before the beginning of the experiments. These data are given in the form of pedigrees. A pedigree extending through four ancestral generations is given for each one of the matings listed in the first column of table 2, or in other words, for all the stock used in the experiments, since, of course, the pedigrees of all the full brothers and sisters in one family will be identical. In these pedigrees I have used the same conventions to indicate ancestral repetition (inbreeding) that have been employed in my earlier papers on inbreeding (Pearl, 19 and later papers in the same series). A solid black circle against an animal's number indicated a primary reappearance, a cross within a circle denotes a reappearance resulting from the fact that some later individual in the ancestral series has been primarily repeated.

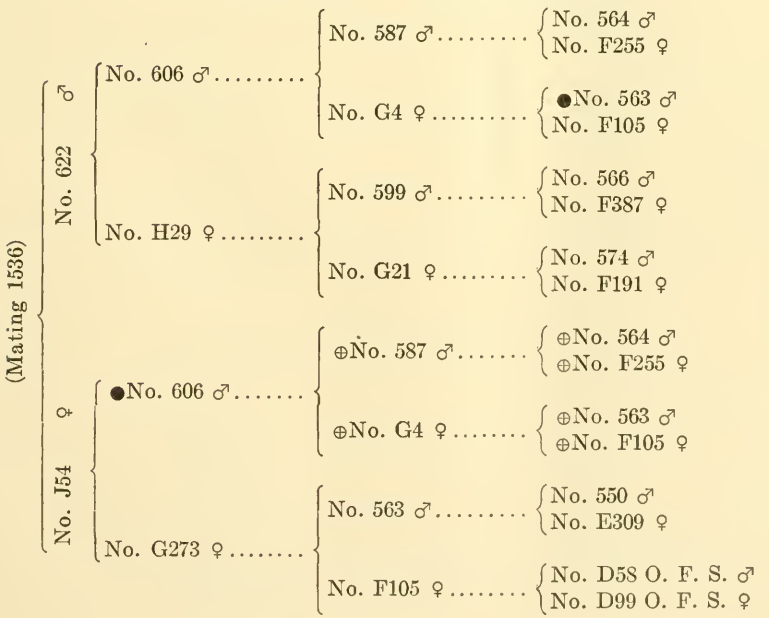
We may first consider the pedigrees of the families furnishing the pure Barred Plymouth Rock females to the alcohol experiments. These pedigrees are arranged in ascending order of mating numbers.

The numbers in the body of the pedigree are bird numbers. The males are designated in nearly all instances by a number alone; the females by a number and a letter. When a bird's number is followed by the letters 'O. F. S.' it is to be understood that this bird formed a part of the original foundation stock with which the writer's poultry breeding experiments started. Information regarding the sources of this original foundation stock will be found in Pearl 17 (p. 137 and 138).

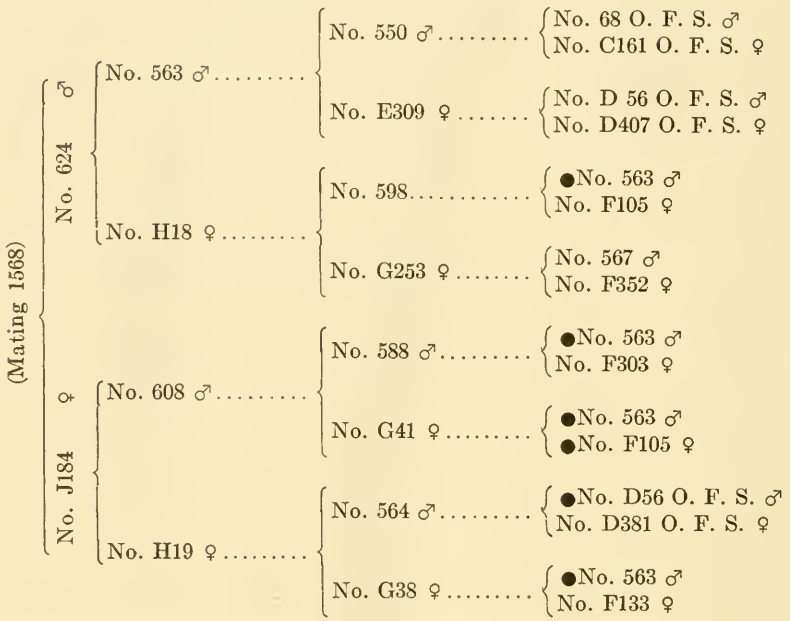
Pedigree of individuals ex mating 1507



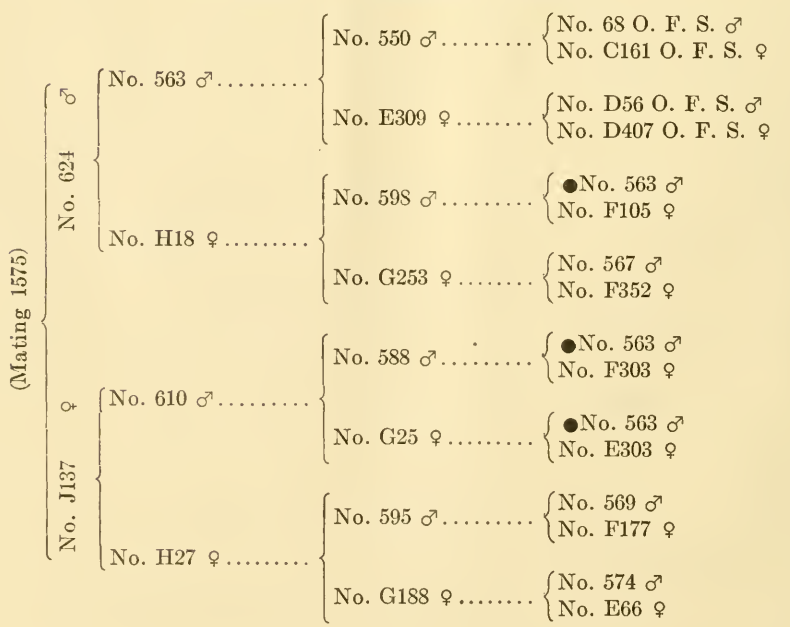
Pedigree of individuals ex mating 1536



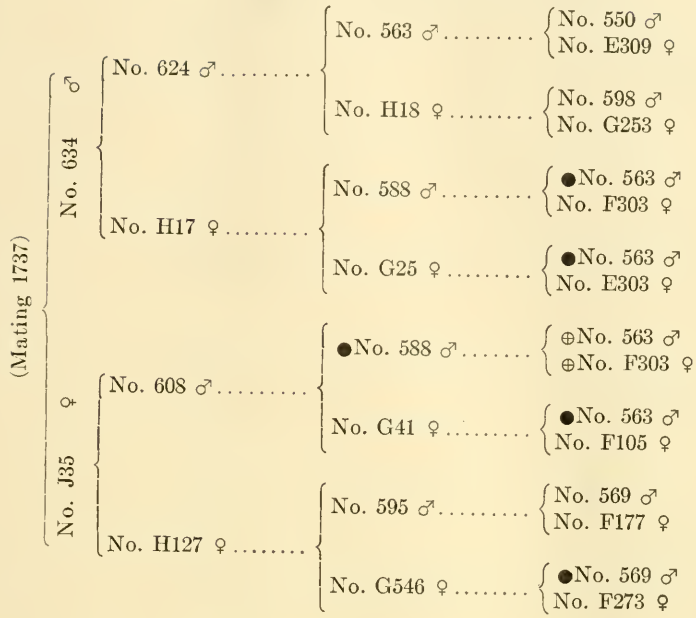
Pedigree of individuals ex mating 1568



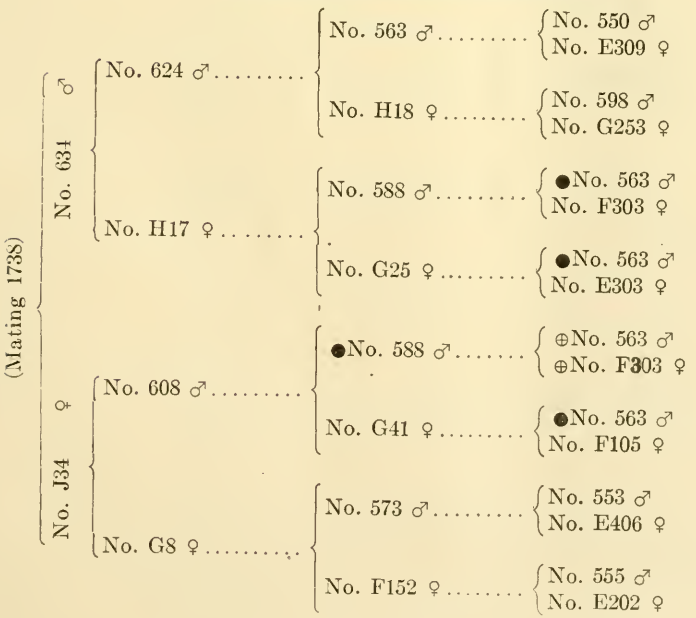
Pedigree of individuals ex mating 1575



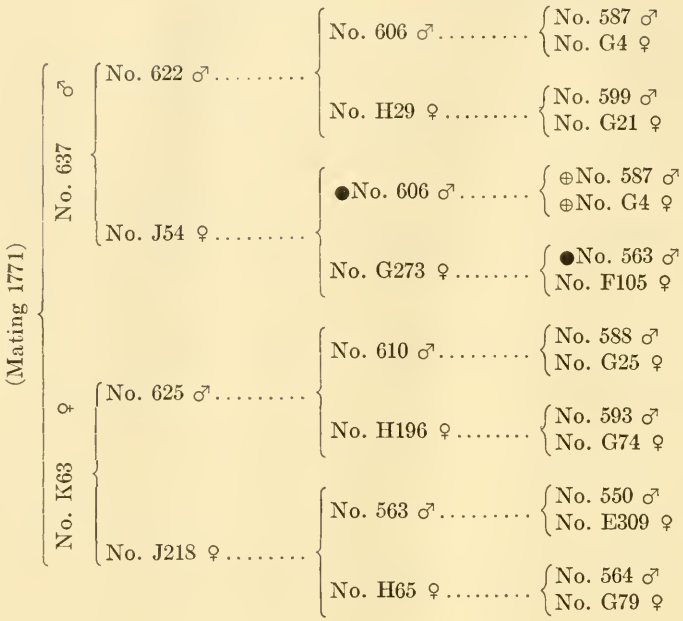
Pedigree of individuals ex mating 1737



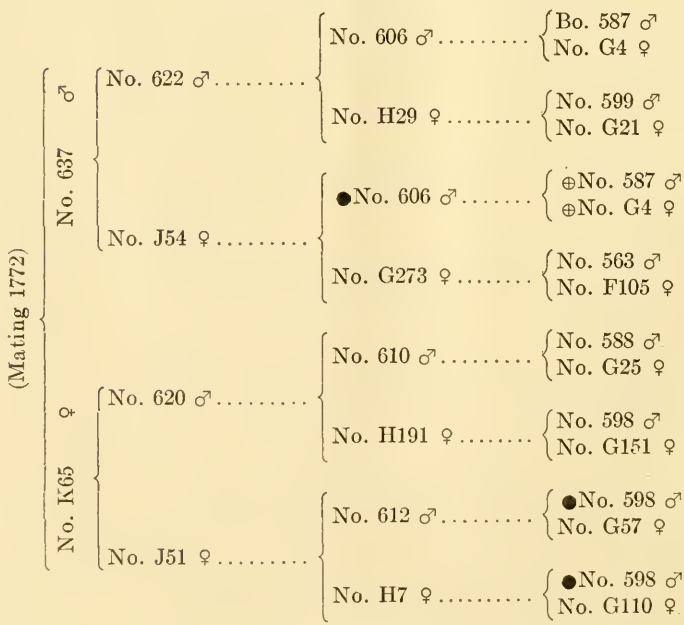
Pedigree of individuals ex mating 1738



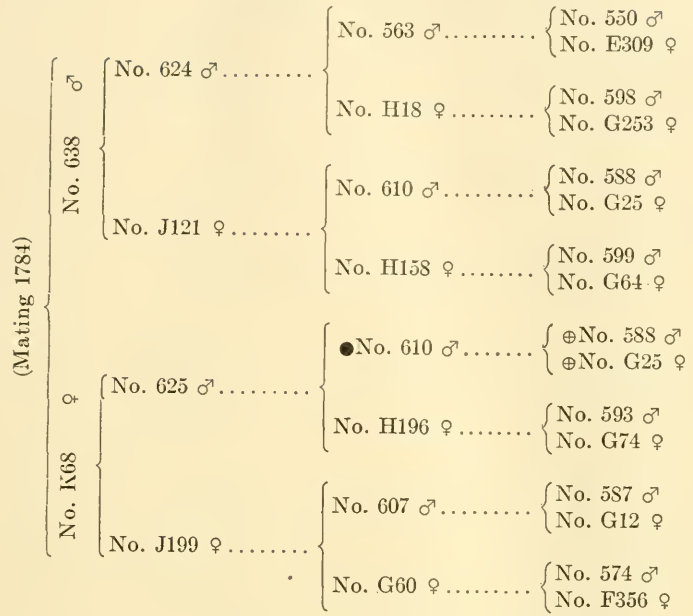
Pedigree of individuals ex mating 1771



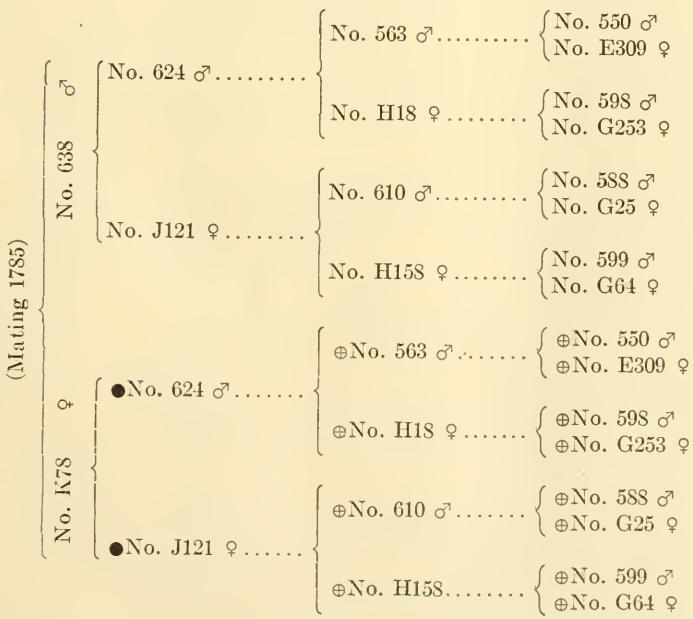
Pedigree of individuals ex mating 1772



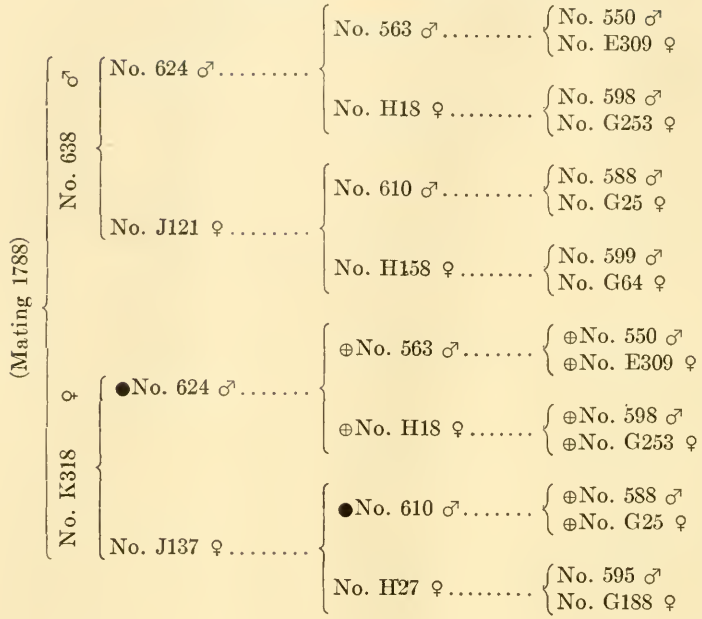
Pedigree of individuals ex mating 1784



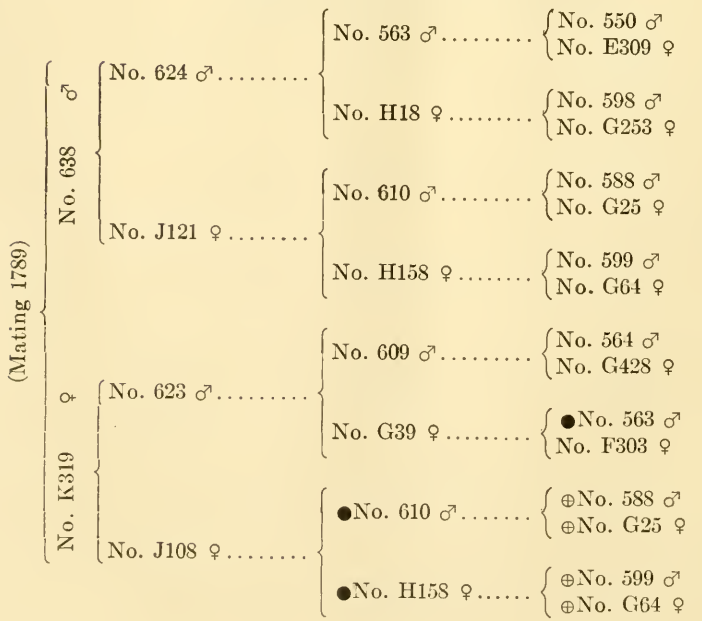
Pedigree of individuals ex mating 1785



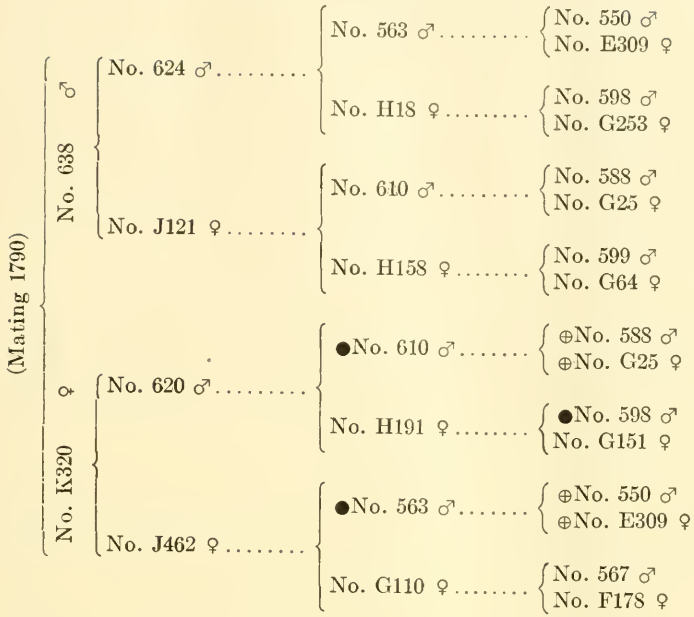
Pedigree of individuals ex mating 1788



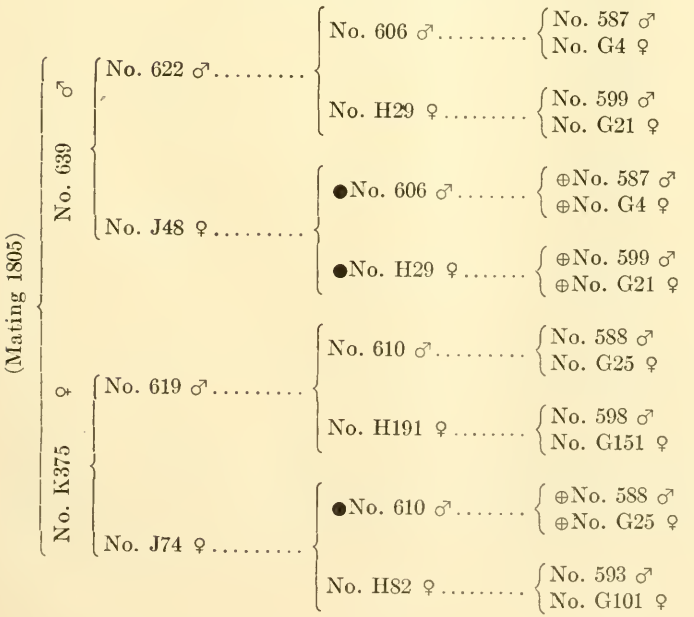
Pedigree of individuals ex mating 1789



Pedigree of individuals ex mating 1790



Pedigree of individuals ex mating 1805



With these pedigrees in hand it is desirable and possible to answer certain questions which arise in the mind of any experienced geneticist in connection with experiments of the sort here under discussion. Some of the more important of these are (a) To what degree is the foundation stock inbred? (b) Does the foundation stock represent a relatively wide or a relatively narrow range of lines of descent (blood lines)? (c) Can the foundation stock be regarded as a fair random sample of the general population from which it was drawn?

Let us consider these points in order. Fortunately we are able, by means of the coefficients of inbreeding devised by the writer, to give precise and definite numerical statements regarding the degree of inbreeding exhibited by this foundation stock. These coefficients are shown in table 3.

TABLE 3

Inbreeding coefficients of stock used in alcohol experiments, with comparison data

MATING	Z_1	Z_2	Z_3
1507	0	0	18.75
1536	25.00	25.00	31.25
1568	0	0	37.50
1575	0	0	18.75
1737	0	12.50	37.50
1738	0	12.50	31.25
1771	0	12.50	18.75
1772	0	12.50	25.00
1784	0	12.50	12.50
1785	50.00	50.00	50.00
1788	25.00	37.50	37.50
1789	0	25.00	31.25
1790	0	25.00	31.25
1805	0	37.50	37.50
Means	7.14	18.75	29.41
Mean of random sample American Jersey cattle	4.10	6.97	12.50
Continued single cousin mating	0	25.00	50.00
Continued parent × offspring mating	25.00	50.00	68.75
Continued brother × sister mating	50.00	75.00	87.50

From the data given in this table it is apparent that this stock cannot, on the average, be considered to be closely inbred. The mean coefficients, except in the case of Z_1, are very decidedly smaller than the coefficients for single first cousin mating. In all the matings except 1536, 1785, and 1788 the value of Z_1 is zero. Two of these are half brother and sister matings and the third is a full brother × sister mating. Omitting these three matings we get for mean values: $Z_1 = 0$, $Z_2 = 13.63$, $Z_3 = 26.14$.

That the mean coefficients of inbreeding for the foundation stock in these experiments should be higher than those for a random sample of the general population of Jersey cows (data from Pearl and Patterson, 25, p. 60) is in no way remarkable when it is recalled that the Barred Plymouth Rock flock of the Maine Station has been line-bred for a long time. It is equally clear that the degree of inbreeding exhibited by the present poultry stock is well below the degree (if there be any such) which, *per se*, causes a weakness and lack of constitutional vigor. This is evident from many considerations. Data which will be presented later on in this paper demonstrate it. In this connection it is of interest to examine a little more closely the performance of the individuals from the most closely inbred mating of table 3. Mating 1785, which was of brother × sister, contributed two individuals, 1482 and 1741, to the mating list of the alcohol experiments. It will be shown in table 1 of paper No. III in this series that 1482, the ethyl treated sister, produced eggs giving a 75.0 per cent hatch on the basis of all eggs set, and 81.8 per cent on the basis of fertile eggs. No. 1741, the untreated sister, did nearly as well. Of all of her eggs set 70.0 per cent hatched; of her fertile eggs 77.8 per cent hatched. In neither of these records can one find any evidence of constitutional weakness, induced by inbreeding or in any other way.

We may summarize the results of our examination of inbreeding in the foundation stock used in these experiments by saying that while it is a sample from a line-bred population and is therefore, to some degree, inbred, the amount or intensity of

this inbreeding is low, on the average, even as compared with single cousin mating.

The data from which an answer may be obtained to our second and third questions are most readily had from an examination of the males which have been used as sires in the Station's Barred Rock flock for a period of years. Since all the females used as breeders within this period will be descendants or collateral relatives of these males, we are able to form an adequate idea of the blood lines involved in our alcohol foundation stock from an examination of the males. In the following résumé the bird number of every male Barred Plymouth Rock used as a breeder in the years 1910–1914 inclusive is given. Each bird number is followed by a figure in brackets. These bracketed figures show the number of times the designated male appears in the first four ancestral generations of the stock used in the alcohol experiments, as shown in the pedigrees exhibited in the preceding pages.

B. P. R. males used as breeders

In 1914

Birds Nos. 634 (2), 636 (0), 637 (2), 638 (5), 639 (1), 640 (0).

In 1913

Birds Nos. 619 (1), 620 (2), 621 (1), 622 (4), 623 (1), 624 (11), 625 (2).

In 1912

Birds Nos. 563 (36[3]), 606 (9), 607 (1), 608 (3), 609 (1), 610 (15), 611 (0), 612 (1), 613 (0).

In 1911

Birds Nos. 563 (36), 564 (6), 587 (10), 588 (20), 589 (1), 593 (3), 595 (3), 597 (0), 598 (16), 599 (12).

In 1910

Birds Nos. D31 (1), 552 (0), 554 (2), 562 (1), 563 (36), 564 (6), 566 (1), 567 (4), 569 (3), 573 (1), 574 (3).

From the above data it will be seen that of the 40 Barred Plymouth Rock males used as breeders in the general population in the years 1910 to 1914 inclusive, thirty-four, or all but six (Nos. 552, 597, 611, 613, 636, and 640) are represented in the

[3] In this and other cases where the same male was used in successive years the number in brackets is the total number of occurrences regardless of generations, just as in the cases where the male was used during one year only.

pedigrees of the stock used in the alcohol experiments. Of these six males not represented some were used chiefly in cross-bred matings and, therefore, left but few pure Barred Plymouth Rock descendants: others were poor breeders and left few descendants of any sort.

It seems evident without further discussion that the Barred Rock stock in the alcohol experiments included practically as many blood lines as the general population from which it was drawn and may be regarded as a representative sample of that population.

The pedigrees of the Black Hamburgs in the experiments may next be considered.

Pedigree of individuals ex mating 1896

Mating 1896 { No. 630 O. F. S. ♂ ; No. K222 ♀ { No. 631 O. F. S. ♂ ; No. 1351 O. F. S. ♀ }

Pedigree of individuals ex mating 1900

(Mating 1900)

No. 1350 O. F. S. ♀ No. 630 O. F. S. ♂

Pedigree of individuals ex mating 1901

(Mating 1901)

No. 1352 O. F. S. ♀ No. 630 O. F. S. ♂

Pedigree of individuals ex mating 1903

(Mating 1903)

No. 1358 O. F. S. ♀ No. 630 O. F. S. ♂

It is evident from these pedigrees that we are much nearer original foundation stock in the case of the Black Hamburgs than with the Barred Rocks. It is important to notice in this connection, however, that these O. F. S. Hamburgs had been previously bred upon the Station plant and their purity and Mendelian behavior thoroughly tested before they were used to breed the stock which was used in the alcohol experiments.

D. Time of beginning treatment

The inhalation treatment was started on the different birds at different dates. This was done in order to determine whether the length of time they had been treated prior to entering upon the breeding season would make any difference in their performance as breeders in the breeding season of 1915. The data regarding the beginning of the experimental treatment of the different birds are set forth in table 4, which requires no further explanation.

TABLE 4

Showing the dates of beginning of alcohol treatment

DATE	TREATMENT BEGUN ON INDICATED BIRDS		
	Ethyl alcohol	Methyl alcohol	Ether
October 1, 1914	♀1574		
October 3, 1914		♀1575	
October 6, 1914			♀1572
October 8, 1914			♀1573
November 6, 1914	♀1482	♀1486	♀1491
December 1, 1914	♀1483, ♂664	♀1487, ♂663	♀1485, ♂665
December 20, 1914			
January 1, 1915	♀1484	♀1492	♀1490
January 15, 1915	♀♀1481, 1489		

E. Plan of matings in 1915

The alcoholized birds and the untreated controls were mated early in February, 1915. Eggs were saved for incubation from these matings from about February 15. The general methods of handling these matings, incubation, brooding the chicks, etc., were the routine methods followed in the writer's breeding experiments with poultry. Long experience has proved these methods to be excellently adapted to the rearing of normal healthy chicks. They are described by Pearl (22).

During incubation and brooding, indeed throughout life, the eggs and chicks from these alcohol experiments were not separated from the general flock. In other words, the eggs from these experiments were put at the same time in the incubators

with eggs from other experiments, being kept separate by wire partitions at the time of hatching for pedigree purposes only; the hatched and properly tagged chicks were indiscriminately mixed with normal chicks from other experiments in the brooders and later in the adult houses. At no stage in the life history have the offspring of alcoholic matings been kept by themselves. By this indiscriminate mixing with the general flock any possibility of conscious or unconscious differential treatment of these birds has been avoided. Any differences which appear between various groups of these F_1 progeny individuals must be attributed to differences antecedent in point of time to their own contact with the environment.

The general plan of the matings in 1915 was to breed a treated male of each of the three classes, ethyl, methyl and ether with (a) untreated control females, and (b) with treated females of his own class (i.e., ethyl ♂ × ethyl ♀, methyl ♂ × methyl ♀, ether ♂ × ether ♀). In addition to these matings an untreated control male was mated with (a) untreated control females, (b) ethyl females, (c) methyl females, and (d) ether females. A general conspectus of all the 1915 matings is exhibited as table 5.

All of the matings were of the type Black Hamburg ♂ × Barred Plymouth Rock ♀.

Each male might have been mated with more females than he was. The matings were purposely kept small in number in order that there could be no possible criticism that the males were overworked in the breeding pens and in consequence produced weak or degenerate offspring.

There was nothing in the sexual behavior of the alcoholized males noticeably different from that of normal males. All three of the treated males used proved to be very vigorous breeders. A characteristic, but not especially significant, bit of behavior was noted in the fact that nearly always the first act of a male bird upon being released from an alcoholic inhalation treatment in the tank was to copulate with one of the females in the pen. It is not entirely certain whether this is to be regarded as an expression of an increase in the libido sexualis

TABLE 5

Plan of matings in 1915

NATURE OF MATING	MATING NUMBER	♂ NUMBER	♀ NUMBER
Untreated ♂ × Untreated ♀	2131	666	1736
	2132	666	1744
	2133	666	42
Untreated ♂ × Ethyl ♀	2127	666	1483
	2128	666	1484
Untreated ♂ × Methyl ♀	2129	666	1492
Untreated ♂ × Ether ♀	2126	666	1485
Ethyl ♂ × Untreated ♀	2116	664	487
	2117	664	1741
	2118	664	1738
	2119	664	1734
Ethyl ♂ × Ethyl ♀	2112	664	1481
	2113	664	1482
	2114	664	1574
	2115	664	1489
Methyl ♂ × Untreated ♀	2123	663	1737
	2124	663	1742
	2125	663	1733
Methyl ♂ × Methyl ♀	2120	663	1486
	2121	663	1487
	2122	663	1575
Ether ♂ × Untreated ♀	2104	665	23
	2109	665	1743
	2110	665	1740
	2111	665	1735
Ether ♂ × Ether ♀	2105	665	1490
	2106	665	1491
	2107	665	1573
	2108	665	1572

induced by the alcoholic treatment, or as simply the normal behavior of any vigorous male that has been kept away from the females for a time. Probably there is something of the former factor involved in the behavior.

F. Germinal dosage index

It is reasonable to suppose that the effect, if any, of the alcoholization of the parents upon the progeny will depend in some degree at least upon the period of time during which the parents

have been subjected to treatment with alcohol prior to the birth of the offspring. This may well be the case even though alcohol is a poison which is comparatively rapidly eliminated from the body and not accumulated, as are some metallic poisons. In planning the present experiments it seemed highly desirable to arrange matters in such way that a fairly wide range of variation in the duration of treatment prior to the hatching of the offspring might be tested. That this point has appealed to other workers in the same field is shown by the following statement in the first report on Stockard's alcohol experiments. Stockard and Craig (37, p. 579) say:

> These experiments have suggested many questions still to be solved, some of which are now being tested. The length of time necessary to treat an animal before the resulting offspring is affected, whether this time is equally long for both sexes, and what amount of individual variation exists.

This phase of the problem appears, however, not to have been followed by Stockard, because one does not find in any of the reports so far published on his work any definite numerical statements regarding the duration of treatment for particular matings before the birth of litters furnishing the data on which the conclusions are based.

In the present investigation the following reasoning has been used in devising a numerical expression of the dosage, so far as concerns the progeny. Two germ cells, a sperm and an ovum, unite to form the zygote of each progeny individual. It is proposed to designate as the 'total germ dosage index' the total number of days during which the two gametes making the offspring zygote have been exposed to alcoholic influence while sojourning in the body of the treated individuals. Such a germ dosage index could, of course, be calculated for each individual progeny chick born. It seems, however, more desirable for present purposes to combine the figures for each mating, and take the sum of the number of days from the beginning of treatment of the male parent to the average date of hatching of the progeny, plus the number of days from the beginning of treatment of the female parent to the average date of hatching

of the progeny as the germ dosage index for that mating. This can be expressed in a formula as follows:

$$\textit{Total germ dosage index in days} = (M_h - A\ ♂) + (M_h - A\ ♀),$$

where

M = Mean date of hatching of progeny.
$A♂$ = Date when treatment of ♂ parent began.
$A♀$ = Date when treatment of ♀ parent began.

It will be seen that this looks at the matter of germinal dosage from the standpoint of the zygote formed by the union of the germ cells. If only one of the germ cells has been exposed to influence by the alcohol it alone will contribute to the total germ dosage index. If, on the other hand, both parents have been treated, both gametes will contribute. This makes it possible to put into one continuous series a numerical expression of the degree of alcoholic treatment for both matings in which

TABLE 6

Total germ dosage index for alcoholic matings in 1915

NATURE OF MATING	MATING NUMBER	MEAN DATE OF HATCHING	TOTAL GERM DOSAGE INDEX
		April	
Ethyl ♂ × Untreated ♀	2119	9	130
Methyl ♂ × Untreated ♀	2125	9	130
Methyl ♂ × Untreated ♀	2123	10	131
Ethyl ♂ × Untreated ♀	2118	14	135
Ether ♂ × Untreated ♀	2111	15	136
Methyl ♂ × Untreated ♀	2124	16	137
Ether ♂ × Untreated ♀	2104	16	137
Ether ♂ × Untreated ♀	2109	21	142
Ether ♂ × Untreated ♀	2110	22	143
Ethyl ♂ × Untreated ♀	2117	26	147
Ethyl ♂ × Untreated ♀	2116	27	148
Ethyl ♂ × Ethyl ♀	2115	7	213
Ethyl ♂ × Ethyl ♀	2112	10	216
Methyl ♂ × Methyl ♀	2121	7	256
Ether ♂ × Ether ♀	2106	15	296
Methyl ♂ × Methyl ♀	2120	27	320
Ethyl ♂ × Ethyl ♀	2113	20	336
Ether ♂ × Ether ♀	2107	27	349
Ether ♂ × Ether ♀	2108	27	351
Methyl ♂ × Methyl ♀	2122	27	354

one of the parents only is treated and matings in which both the parents are treated.

Table 6 gives in order the germinal dosage index for each of the F_1 matings which produced offspring in these experiments. The matings are arranged in ascending order of total germ dosage index.

From this table it is seen that the total germ dosage index for the F_1 progeny in these experiments ranges from 130 days to 354 days with the matings for the different substances used well scattered over the range. The facts are represented graphically in figure 1 of paper No. III in this series.

G. Scope of present reports

It is the purpose of the present paper and the two next following in this series to present and discuss the data which have accumulated in this investigation from September, 1914 up to February 1, 1916. This includes the F_1 generation of progeny only. The experiment is of course being continued and later reports will be given on further generations of progeny and others matters of interest not taken up in the present reports.

III. SUMMARY

This paper is the first of a series of studies having to do with attempts, in the first place, to modify hereditary factors or determinants in a definite and specific way, and in the second place, to observe and analyze the hereditary behavior following such modification. The results here reported followed attempts to modify the germ cells by treating the individual domestic fowl with one or another of three poisons, viz., ethyl alcohol, methyl alcohol, and ether.

Summarily stated the chief points brought out in the paper are:

1. The males used in the experiments were pure bred Black Hamburgs. The females were pure bred Barred Plymouth Rocks. There are shown to be numerous advantages in having the progeny of treated parents F_1 crossbreds rather than pure.

2. A detailed account of the breeding of the stock used, prior to the beginning of these experiments, is given. It is shown to be inbred to only a comparatively low degree. It is shown to be a random sample of the general population from which it came. Full brothers and sisters of treated are used as controls.

3. The poisons used were administered daily by the inhalation method in practically as large doses as could be tolerated when given in this way. An account of the methods used and the precautions taken to ensure critical results will be found on pp. 132–138.

4. The total germ dosage index, defined as the total number of days to which the gametes forming zygotes had been exposed to treatment when the offspring were produced, ranged from 130 to 354 days in these experiments, with a mean of 210.35 days, or approximately 7 months.

The results of these experiments will be presented in the two next following papers in this series. Those two papers and the present one (Nos. I, II, and III in the series) deal with the results from the beginning of the alcohol experiments in September, 1914 up to February 1, 1916. Later reports will deal with the results after the latter date.

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THE EXPERIMENTAL MODIFICATION OF GERM CELLS

II. THE EFFECT UPON THE DOMESTIC FOWL OF THE DAILY INHALATION OF ETHYL ALCOHOL AND CERTAIN RELATED SUBSTANCES[1]

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FOUR FIGURES

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I. INTRODUCTION

Before entering upon any discussion of the effect of the alcohol treatment on the progeny it seems desirable to examine with some care into the effects, both structural and physiological, upon the treated individuals themselves of the daily administration by the inhalation method as described in I,[2] of ethyl alcohol, methyl alcohol, or ether. In this examination attention will be confined to characters which are capable of quantitative definition and measurement. It seems highly desirable in the experimental study of a matter so warmly debated to deal chiefly with things which can be measured.

A limitation of the present section of this report is found in the fact that the experiment is still in progress and only a small

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station. No. 101.

[2] This refers to the first paper in this series, which was entitled: "The experimental modification of germ cells. I. General plan of experiments with ethyl alcohol and certain related substances," Jour. Exp. Zoöl., vol. 22, pp. 125–164. Throughout this and later papers in the series cross-references to other papers in the same series will be made simply by the Roman numeral designating the paper referred to, together with the particular page number to which reference is made.

amount of exact autopsy material from treated individuals has as yet come into hand. Later it will be possible to deal more exhaustively with organ weight data.

It will be recalled that, as stated in I, the present report includes only the data obtained from the beginning of the experiment in September, 1914, up to February 1, 1916.

II. MORTALITY

Inasmuch as many of the birds with which the experiment started are still alive it is obviously impossible at the present time to go into the question of the effect of the alcohol treatment upon the duration of life. What can be done, however, is to examine the facts as to the mortality of the treated and control birds during the first 15 months of the experiment. The data for such an examination have already been given in table 2 of I for the females only, and are summarized in convenient form in table 1 of the present paper.

In explanation of the headings of table 1 it should be said that considerations of space on the poultry plant made it necessary to dispose of a part of the control birds (untreated) in the fall of 1915. The individuals so disposed of by sale were nearly a random sample from every point of view of the respective matings from which they came. They were certainly a random sample so far as concerns general bodily vigor and probable duration of life. From the standpoint of mortality figures the only thing which can be said of them, however, is that they are certainly known not to have died within the first 500 days of their lives. It should be further noted in regard to table 1 that in the case

TABLE I

Showing data regarding mortality to February 1, 1916. Females only

CLASS	NUMBER AT BEGINNING OF EXPERIMENT	SOLD AT END OF PULLET YEAR	KILLED OR DIED BY ACCIDENT	DIED OTHERWISE	LIVING FEB. 1, 1916	NET MORTALITY PER CENT
Treated.....	15	0	7	0	8	0
Untreated controls...	39	14	1	16	8	41.0

of matings 1568, 1536 and 1575 only such untreated control birds are included in any column of the table as were on the plant at the beginning of this alcohol experiment. In other words, no birds from these matings which were sold in the fall of 1914, or died before that time, are here regarded as having been in the experiment, even as controls. For some other purposes these birds may be, and are used as controls.

From this table it appears that:

1. Out of the 15 treated birds with which the experiment started the only ones which had died at the end of 15 months were those which were killed by an overdose of the reagents used (cf. I, p. 166).

2. Out of 24 (=39–15) untreated control sisters of the treated birds, which started in at the same time and have been kept on the plant until they died or to the present time, 16 have died, or 41.0 per cent of the whole number which started or 66.7 per cent of the 24 which were given every opportunity to live through the experiment if they were able to do so.

It is obvious from these figures, if we take them at their face value, that the mortality so far has been much heavier among the untreated control birds than among the treated. There is nothing which would gainsay such conclusion to be found from an examination of the causes of death of the 17 control birds which died. The pertinent autopsy data in this connection are given in table 2. The diagnoses are based on symptoms and lesions described in detail by Pearl, Surface and Curtis (30. This and other citation numbers in this paper refer to the bibliography at the end of I).

From this table it is seen that out of the 16 deaths from non-accidental causes 9 were due to diphtheria or diphtheritic roup, either with or without other complications; 2 were due to visceral gout; 2 had their original causes in derangements of the oviduct; and finally diarrhea, pernicious anemia, and peritonitis, probably not of oviducal origin, each caused one death in the group.

Roup has existed in endemic form on the Maine Station Poultry plant for many years, as on most other plants where for experimental, or any other purpose, birds are brought in from outside

TABLE 2

Causes of death in control females. Data to February 1, 1916

BIRD NO.	AUTOPSY NO.	CAUSE OF DEATH
M487	1045	Diphtheritic patches in larynx and trachea. Pneumonia patches in lungs.
372	938	Visceral gout.
69	984	Peritonitis. Derangement of oviduct as initial cause.
1508	1031	Lungs congested. Diphtheria patches in throat.
K42	969	Large concrement in oviduct.
M36	991	Choked to death in trapnest. Accidental death.
1737	1177	Diphtheritic roup.
506	977	Digestive disorders. Diarrhea.
1744	1198	Diphtheritic roup.
M24	998	Diphtheria.
1736	1203	Diphtheritic roup.
118	949	Pernicious anaemia.
440	1006	Roup—some peritonitis.
365	943	Visceral gout.
1724	1145	Diphtheritic roup.
1671	1121	Diphtheria.
1549	1037	Peritonitis.

fairly frequently. Ordinarily it gives very little trouble. Occasionally it will break out into an epidemic of greater or less violence, always as a result of a relaxation of some routine sanitary or hygienic measure. During the course of this alcohol experiment we have passed through a particularly violent epidemic of the sort mentioned. This fact is reflected in the large proportion of the deaths due to diphtheritic roup or some of its complications. On account of this epidemic the total mortality in the experiment must be regarded as abnormally high. The remarkable thing is that during the 15 months covered in this report, i.e., to February 1, 1916, not a single one of the treated birds succumbed to this disease, though they were exactly as much exposed to contagion as the controls. This is a surprising result. It seems impossible that it can be due to any real increase in resisting power in the alcoholic birds. A possible explanation is that the daily inhalation treatment acts as a disinfectant of the air passages, and the treated birds do not take the disease because its germs are killed or greatly weakened before thay have an opportunity to get an effective foothold. It

would be altogether premature to draw any conclusion in regard to the matter until more extended data are at hand. At present I desire merely to put on record the facts now available.

During the period covered by the present report none of the male birds, either alcoholic or control, died.

The superior mortality record of the treated birds, while a side issue to the main genetic interest of the study, has some interest on its own account in connection with the general problem of the effects of alcohol upon the organism. There is a widespread popular opinion that life insurance statistics have 'proved' that even the most moderate use of alcohol definitely and measurably shortens human life. In common, as I suppose, with most persons who have made no special personal investigation of the original literature on the subject I had supposed this statement to be true. The present results were, however, so clear-cut in the opposite direction that my curiosity was aroused to examine critically the actuarial evidence. The results were somewhat astonishing. The evidence on which the current statements are based would not be accepted by anyone trained in the critical valuation of statistical and biological evidence as 'proving' anything. All of the various actuarial investigations of the question which have been made, including Moore's analysis of the experience of the United Kingdom Temperance and General Provident Institution, McClintock's review of the experience of the Mutual Life of New York, Phelps' study of the experience of the Northwestern Mutual Life, and the widely quoted Medico-Actuarial Mortality Investigation, based on the mortality experience of 43 American life insurance companies, appear to suffer, in greater or less degree, from the following defects, which entirely invalidate them for the purpose of determining critically and scientifically the effect of alcohol in different dosages upon human longevity: (1) The numbers dealt with are small. (2) There is no evidence of any sort or kind as to how much alcohol the subjects of the investigations consumed except their own statements on the subject made at the time insurance was applied for. (3) No allowance is, or can be, made for the influence of almost numberless other factors which may differentially influence the mortality in the groups com-

pared. (4) There is no control on the question of whether the drinking habits of the insured changed during the life of the policy. I am informed by competent actuarial experts that they regard the problem of the effect of the moderate use of alcohol (corresponding to the dosages employed in the present investigation) upon human longevity as still an absolutely open question.

In this same connection the statements of Heron (10) regarding the death rate among extreme alcoholists is of interest. He made a very thorough and critical investigation of the mortality and morbidity of female inebriates, committed under the Inebriates Act between January 1, 1907, and December 31, 1909, to the inebriate reformatories in England. He first shows in detail what must be evident on general grounds, that any person to come under the operations of the act and be committed to a reformatory must be chronically, extremely, and, as the event shows, practically incurably addicted to the regular and excessive use of alcohol. They represent the upper limit of chronic alcoholism. In regard to morbidity he finds (p. 17) that 77 per cent of these maximally alcoholic persons "are free from definite organic disease." Regarding mortality he finds (p. 22)

> that the death-rate from all causes among inebriates while under sentence is only half that of the total female population of England and Wales and is less than a fourth of the death-rate of the class from which they are drawn, if the assumptions made in arriving at the death-rate among this class be accepted; the death-rate among inebriates from cancer is slightly less and from phthisis is decidedly less than in this class. The lower death-rate from phthisis is possibly due, to some extent at least, to selection before admission and close medical supervision after admission to the Reformatories.

The official German statistics show in general a smaller death rate from tuberculosis among alcoholists than among abstainers. This result, which is similar to that obtained by Elderton and Pearson (7), is attributed by Pearson (31, p. 16) to selection, in the manner that individuals of better physical constitution are more likely to be drinkers. Such a factor as this would, of course, not come into consideration in the experiments with fowls at all.

III. BODY WEIGHT

Stockard and Papanicolaou (38, p. 72) make the following statement regarding their guinea pigs:

> The general condition of the animals under the fume treatment is very good. They all continue to grow if the treatment is begun before reaching their full size, and become fat and vigorous, taking plenty of food and behaving in a typically normal manner. . . . . Alcoholized animals are usually fat, but there is no fatty accumulation in the parenchyma of any of the organs.

They give no exact data regarding body weights.

In planning the experiments it was felt to be highly important to collect exact information on the changes in body weight which occurred. Clinical experience has abundantly demonstrated that body weight changes, in spite of their rough and inexact character, furnish an index of general metabolic conditions and changes which is by no means to be despised. Its value is greatly enhanced if parallel data from proper controls are also at hand. The treated birds in these experiments have been weighed at intervals of about one month, except that no records are at hand for October 1, 1915. Owing to an unfortunate misunderstanding the control birds were not weighed as frequently as the treated birds.

The available data on body weight in grams are given in table 3, and figures 1 and 2.

From table 3 and the diagrams the following points are to be noted:

1. The majority of the birds used in the experiment had plainly not completed their growth at the time the experiment started. This is shown by the fact that even with those birds whose treatment did not begin until December 1 or January 1 the same rise in body weight is shown in the earlier months as for those whose treatment began in November (cf. table 4 in I). That this initial rise in the body weight curves can not be due entirely to growth is proven, however, by the fact that ♀ ♀ 1572, 1573, 1574 and 1575 show it just as clearly as do any of the other birds. But these four birds were nearly a year

and a half old at the beginning of the experiment, and their increase in body weight at this time can not be attributed to growth in the ordinary sense of the term. Examination of rather extensive statistics on body weight changes in poultry indicates that there is some tendency for a bird to increase in weight

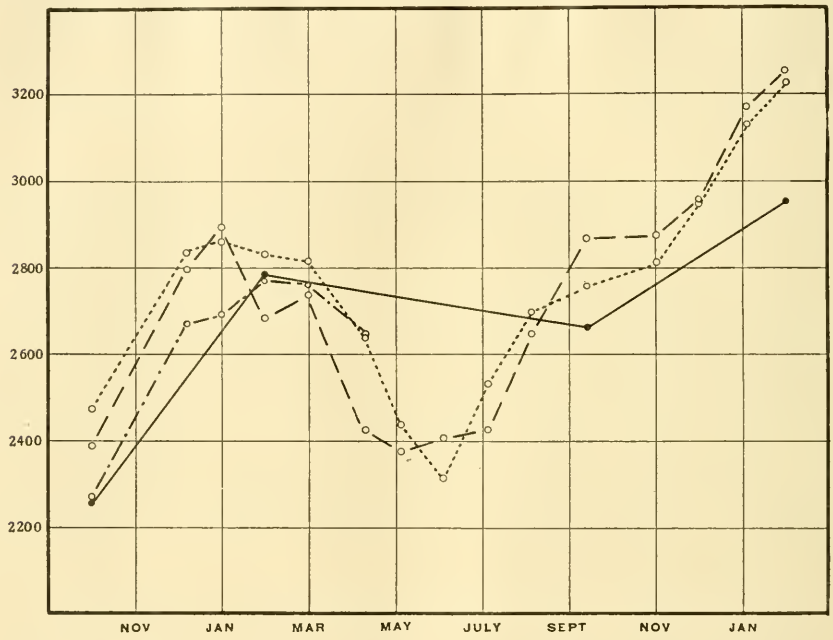

Fig. 1 Diagram showing the changes in body weight of females subject to inhalation treatment. The figures plotted are the means for the specified sorts of birds, except where a statement to the contrary is made. Solid line, untreated controls; dash line, ethyl alcohol birds; dotted line, methyl alcohol birds (here the plottings after September 1915 are based on a single bird); dash-dot line, ether birds.

during the autumn and early winter months, quite regardless of her age. I hope later to be able to present exact and comprehensive data on the normal seasonal fluctuations in the body weight of hens. In the absence, at the present time, of such data, and because of the fact shown by the data given here that this initial rise in body weight in the autumn of 1915 is only slightly less in the controls than in the treated birds, it seems

TABLE 3

Showing the changes in body weight with continued treatment

BIRD NO. AND TREATMENT	BODY WEIGHT IN GRASS AT SPECIFIED DATES														
	October 1, 1914	December 6, 1914	January 1, 1915	February 1, 1915	March 1, 1915	April 10, 1915	May 4, 1915	June 3, 1915	July 4, 1915	August 4, 1915	September 2, 1915	November 2, 1915	December 1, 1915	January 3, 1916	February 1, 1916
Females															
1481, ethyl alcohol	2087	2440	2610	2360	2570	2000	2087	1996	2177	2495	2722	2994	3084	3402	3493
1482, ethyl alcohol	2404	2870	2940	2860	2740	2520	2359	2449	2495	2812	2994	2903	3175	3493	3493
1483, ethyl alcohol	2767	3280	3520	2650	3200	3080	3062	3130	3175	3402	3674	3719	3674	4082	4264
1484, ethyl alcohol	2313	2370	2420	2250	2020	1880	1724	1769	1724	1814	1996	1860	2087	2268	2359
1489, ethyl alcohol	1814	2720	2790	2790	2740	2260	2268	2359	2313	2449	2631	2495	2631	2268	2767
1574, ethyl alcohol	2948	3110	3090	3210	3150	2810	2767	2722	2722	2903	3175	3266	3084	3493	3130
Mean of all ethyl birds	*2389*	*2798*	*2895*	*2687*	*2737*	*2425*	*2378*	*2404*	*2434*	*2646*	*2865*	*2873*	*2956*	*3168*	*3251*
1486, methyl alcohol	2359	2890	2690	2590	2380	2220	2313	2132	2359	2449	2631				
1487, methyl alcohol	2223	3010	2950	2920	3000	2860	2722	2313	2404	2631	2812				
1492, methyl alcohol	2720	2720	2970	2840	2880	2640	2132	2268	2722	2948	2858				
1575, methyl alcohol	2585	2720	2830	2970	2998	2830	2585	2540	2631	2767	2722	2812	2948	3130	3221
Mean of all methyl birds	*2472*	*2835*	*2860*	*2830*	*2814*	*2638*	*2438*	*2313*	*2529*	*2699*	*2756*				
1485, ether	2177	2690	2700	2710	2550	2570	2268	2268	2359	2585	2722	3175	3266	3447	3402
1490, ether	1814	2320	2390	2210	2150	2140									
1491, ether	2132	2220	2250	2230	2350	2250									
1572, ether	2472	2990	3190	3300	3440	3100									
1573, ether	2767	3110	2930	3390	3310	3170									
Mean of all ether birds	*2272*	*2666*	*2692*	*2768*	*2760*	*2646*									
Mean of all untreated controls	*2254*			*2785*[3]							*2658*				*2953*[4]
Males															
♂ 664, ethyl alcohol		1200	1550	1690	1690	1630	1588	1542	1678	1860	1950	2041	2087	2223	2223
♂3257, ethyl alcohol		970	1190	1410	1490	1500	1497	1406	1497	1588					
♂ 663, methyl alcohol		1370	1570	1580	1520	1350	1361	1361	1588	1724	1724	1814	1905	1996	2041
♂ 665, ether		1400	1670	1810	1860	1800	1860	1724	1678	1814	1905	2087	2087	2132	2107

[3] This mean is based on weighings made on January 9, 1915.

[4] This mean is based on weighings made on January 23, 1916.

reasonable to conclude that the inhalation treatment had very little if anything to do with causing it.

2. The male birds show the same initial rise in body weight as do the females. Unfortunately we have no control figures for the males, but in all probability the same considerations hold for them as for the females just discussed.

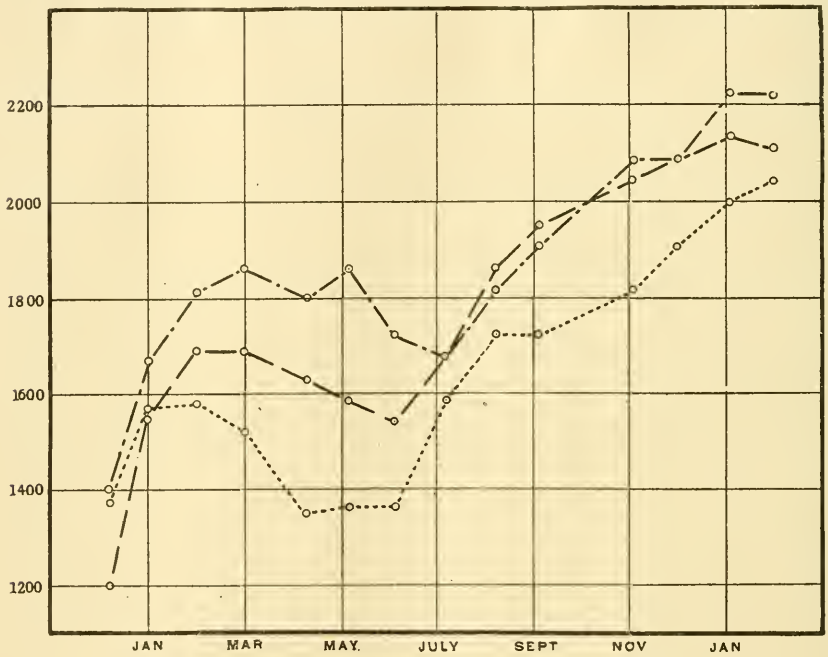

Fig. 2 Diagram showing the changes in body weight of males subjected to the inhalation treatment. The lines have the same significance as in figure 1. All plottings are on the basis of single individuals not means.

3. Following the initial rise, which reaches its peak in January or February, there is a sharp and prolonged fall in body weight which reaches its lowest point in either May or June, in the case of the females and a month or two months later in the case of the males 664 and 665. The absolute amount of this loss in body weight is large, particularly in the females. In it is probably to be seen the first significant effect of the continued administration of the poisons. Just as before, it is im-

possible to measure exactly the portion of this effect resulting from the treatment because of the absence of weighings of untreated controls during this critical period. There is probably normally some decline in body weight between January and June. It is, however, not normally as great as that here observed in the treated birds.

4. Beginning in June, 1915 in the case of the ethyl alcohol birds, and in July, 1915 in the case of the methyl birds, there has been a steady increase in the mean weight of the treated birds up to and including February 1, 1916. At the latter date the treated averaged about 300 grams more per bird in body weight than the controls. Put in another way, the alcohol birds were 9.9 per cent heavier after about 15 months of inhalation treatment than untreated control birds of the same average age. This is a sensible, though not very great difference. It certainly does not indicate that any profound or far-reaching effect upon the general metabolic processes of these birds has as yet been produced by the treatment.

5. In general the changes in the body weight shown by treated Barred Plymouth Rock females are paralleled in the treated Black Hamburg males. The chief difference is that the changes are absolutely somewhat smaller in the males.

The further course of the body weight changes in these birds will be watched with great interest. In such birds as have so far come to autopsy there has been no indication of fat infiltration of any of the visceral organs. Apparently the 10 per cent increase in weight is due entirely to deposition of body fat.

IV. EGG PRODUCTION

In the egg production of fowls we are dealing directly with an easily measurable activity of the very organ whose products we hope to influence, namely the ovary. On this account it is of particular importance to examine carefully the facts regarding this character in the alcoholized birds as compared with their untreated sisters. The present experiments may be regarded as especially favorable for such a study, inasmuch as the Barred Plymouth Rock females used in the work had been

pedigree bred for a number of years and their hereditary qualifications in respect of egg production were well known (Pearl, 18). I have lately called attention (23, p. 134) to some of the difficulties which are involved in interpreting critically and fairly the results of physiological experiments in which egg production is used as an indicator. One must have proper controls in the first place. The ideal condition in respect of controls is only reached when one has full sisters of the experimentally treated birds. This condition has been met in the present experiments. In the second place the management of the birds in the experiment should be such that one can be sure that the control birds are laying normally throughout the experiment. By normal laying in this connection is meant the full somatic expression of the innate inherited capacity of the birds for egg production. Unless this is reached by the controls in any physiological experiment on egg production one never can be quite sure that any difference which may appear between the control and experimentally treated birds is not due to the effect of some overlooked environmental factor upon the controls, which reduced their production below what it should have been.

The egg production of all the birds in these experiments, by months, is given in table 4. In this table 'D' means that the bird in question died in the indicated month after laying the number of eggs in that month shown by the figures preceding the D.

From this table it is evident that all of the birds, both treated and untreated, have laid normally throughout the experiment. To get beyond this general impression and make exact comparisons between controls and treated birds it is necessary to reduce the mass of figures of table 4 to means or averages. This has been done in table 5. In calculating the means in table 5 only such birds have been included as began their first laying year at the beginning of the experiment, namely the autumn of 1914. Those birds which were yearling hens at that time (treated birds numbered in the 1500's and their sisters) were entering their second laying year. First year and second year laying records are not homogeneously comparable, and therefore should

TABLE 4

Monthly egg production of treated and control birds

BIRD NO. AND TREATMENT	1914			1915												1916	TOTAL
	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	
1481, ethyl		*8*	*27*	*12*	*17*	*23*	*12*	*22*	*19*	*19*	*19*	*17*	*13*	*18*	*2*	*13*	241
1483, ethyl		*8*	*21*	*6*	*0*	*19*	*21*	*22*	*21*	*20*	*14*	*12*	*3*	*0*	*5*	*4*	176
1726, untreated	10	17	21	23	21	24	22	26	20	26	23	6	0	12	5	0	256
M364, untreated	1	24	18	18	17	21	9	19	20	19	17	Sold					
487, untreated		1	10	16	19	14	22	22	19D								
1482, ethyl		*0*	11	*18*	*19*	*19*	*20*	*22*	*19*	16	*8*	*6*	*0*	*0*	*2*	*8*	158
1741, untreated	0	0	4	19	4	18	11	15	19	17	17	14	7	4	2	0	151
1484, ethyl		*11*	*16*	*10*	*11*	*17*	*19*	*20*	*21*	*21*	*21*	*20*	*17*	*17*	*7*	*13*	241
69, untreated	21	14	3	0	Dead												
1489, ethyl		*0*	*0*	*14*	*18*	*17*	*14*	*18*	*12*	*14*	*15*	*18*	*21*	*17*	*4*	*0*	182
331, untreated	8	20	8	5	18	21	20	9	17	20	21	Sold					
346, untreated	0	0	0	0	0	22	22	17	13	16	17	Sold					
1738, untreated	0	0	2	15	16	21	22	21	11	15	18	19	21	6	0	0	187
296, untreated	0	0	0	0	9	23	21	17	15	25	14	Sold					
292, untreated	0	0	13	22	21	24	18	1	14	12	14	Sold					
1574[5], *ethyl*	*0*	*0*	*0*	*0*	*6*	*13*	*16*	*21*	*17*	*14*	*2*	*0*	*0*	*0*	*0*	*0*	89
1508[5], untreated	18	11	5	0	1	18	16	17	Dead								
K42, untreated	0	0	0	0	Dead												

TABLE 4—Continued

BIRD NO. AND TREATMENT	1914			1915											1916	TOTAL
	October	November	December	January	February	March	April	May	June	July	August	September	October	November	December	January	
1486, methyl	*0*	*0*	*16*	*21*	*20*	*21*	*17*	*16*	*12*	*8*	*12*	Dead					
M36, untreated	13	13	10	17	18	19D											
348, untreated	0	0	0	0	11	23	22	6	10	5	3	Sold					
1737, untreated	0	0	1	20	19	21	19	21	18	22	18	17	18	11	0	Dead	
1487, methyl		*0*	*14*	*11*	*19*	*21*	*18*	*21*	*19*	*18*	*10*	Dead					
1742, untreated	0	0	16	21	17	18	17	12	19	18	15	12	7	6	0	5	183
65, untreated	0	7	17	14	16	13	5	12	9	0	0	Sold					
1683, untreated	0	11	8	9	22	25	22	24	18	19	21	15	6	0	4	14	218
370, untreated	0	0	0	0	9	11	21	22	18	22	19	Sold					
506, untreated		0	8	19	14D												
1492, methyl		*0*	*17*	*2*	*7*	*20*	*12*	*11*	*11*	*8*	*14*	Dead					
49, untreated	0	17	19	20	18	19	6	17	8	13	1	Sold					
519, untreated		0	0	2	13	19	18	17	21	21	21	Sold					
1744, untreated		0	0	5	17	21	12	18	17	12	22	21	20	15	0	0	180
1575,[5] methyl	*0*	*0*	*0*	*0*	*2*	*15*	*19*	*17*	*17*	*16*	*7*	*0*	*0*	*0*	*0*	*0*	93
1485, ether		*3*	*15*	*14*	*11*	*14*	*15*	*23*	*18*	*10*	*6*	*1*	*0*	*0*	*0*	*0*	130
M24, untreated	21	16	0	13	16	2	5D										
1736, untreated	0	2	0	0	11	16	19	23	24	11	28	21	20	10	0	0	185
118, untreated	9	3	Dead														
440, untreated	0	7	19	10	6	21	17D										

1490, ether		*0*	*5*	*11*	*12*	*23*	*9*D										
1743, untreated	0	2	0	13	18	20	16	14	21	6	16	2	14	10	0	0	152
359, untreated	0	6	21	19	18	12	14	12	21	14	11	Sold					
365, untreated	0	Dead															
1491, ether		*11*	*22*	*17*	*20*	*21*	*12*D										
1724, untreated	25	16	0	21	21	26	25	26	12	22	21	20	9	0	0	Dead	
60, untreated	12	7	0	9	10	18	18	19	17	16	10	Sold					
55, untreated	5	23	18	19	18	23	22	22	18	8	1	Sold					
1671, untreated	0	14	18	16	7	23	24	24	22	23	23	22	21	3	0	Dead	
1740, untreated	0	8	18	17	9	23	22	21	22	21	18	15	16	2	1	2	215
516, untreated		0	1	18	20	20	21	22	20	21	14	Sold					
1572,[5] *ether*	*0*	*0*	*0*	*0*	*0*	*11*	*10*D										
1573,[5] *ether*	0	0	0	0	8	13	9D										
1511,* untreated	13	0	0	9	14	15	9	6	13	0	0	Sold					
1540,* untreated	17	9	5	11	7	14	19	21	18	14	19	18	16	3	0	0	191
1549,* untreated	16	12	12	2	8	12	1	7	Dead								

[5] It should be remembered that these birds were entering upon their second laying year in October 1914. Their records are not comparable directly with those of pullets, cf. Table 2 of I.

not be lumped together in calculating means or other constants. So few of the yearling hens with which the experiment started lived through that there is not enough material to make any critical detailed study of their production. Accordingly we may confine our attention to the laying activity of the pullets with which the experiment started.

In the first column of table 5 are given the monthly mean productions for all treated birds surviving through the indicated

TABLE 5

Mean monthly egg production of treated and control birds.

MONTH	TREATED	N	GENERAL CONTROL	N	SPECIAL CONTROL	N
November, 1914.....	*3.73*	11	6.91	33	6.91	33
December.....	*14.91*	11	7.91	32	7.91	32
January, 1915.....	*12.36*	11	12.50	32	12.50	32
February.....	*14.00*	11	14.63	30	14.63	30
March.....	*19.54*	11	19.48	29	19.54	29
April.....	*16.44*	9	18.15	27	17.26	19
May.....	*19.44*	9	17.74	27	16.79	19
June.....	*16.89*	9	17.08	26	16.17	18
July.....	*13.78*	9	16.35	26	16.28	18
August.....	*13.22*	9	15.50	26	16.06	18
September.....	*12.33*	6	15.33	12	15.00	4
October.....	*9.00*	6	13.25	12	12.00	4
November.....	*8.67*	6	6.58	12	8.00	4
December.....	*3.33*	6	1.00	12	1.75	4
January, 1916.....	*6.33*	6	2.33	9	0	4
Totals.....	*183.97*		184.74		180.80	

month, beginning with November, 1914, and continuing through January, 1916. No bird which died in the course of the experiment is included in the calculation of the mean production for the month in which she died. In the third column of the table, headed 'General Control,' are given the monthly mean productions for all untreated control birds surviving through the indicated month. This column represents the distribution of the production of the general control flock, regardless of whether the treated sisters of any of these control birds had died. In the fifth column of the table, headed 'Special Control,' are given

the mean monthly productions for the full sisters, and only those of the treated birds used to calculate the mean for any month in column 1. In other words, when the ether birds, for example, dropped out by death in April, 1915, all of the untreated control sisters of the ether birds killed were also dropped in calculating the means for the 'Special Control' column. This column gives a critically fair comparison with the treated birds

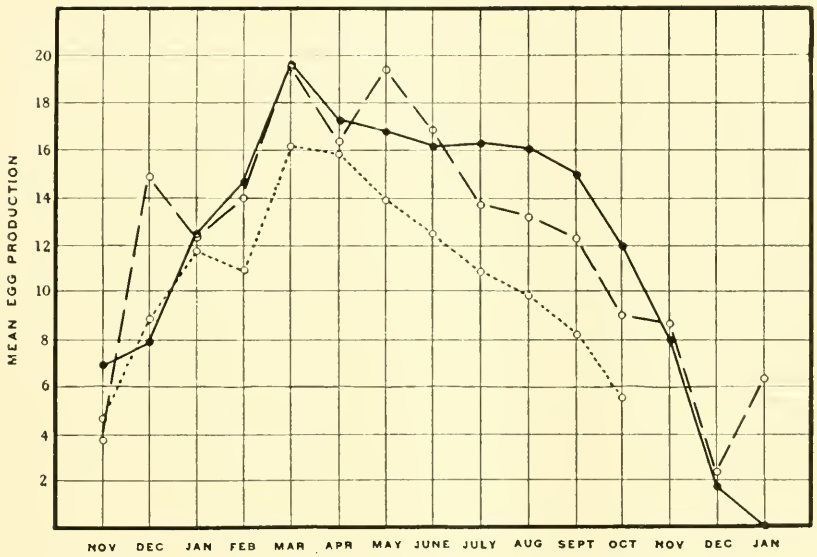

Fig. 3 Showing the mean monthly egg production during the first 15 months of the experiment. Solid line, untreated controls (data from 'Special Control' column of table 5); dash line, all treated birds; dot line, old general flock data (see text).

at any stage of the experiments. The columns headed 'N' give in each case the number of surviving birds on which the calculation of the mean was based.

The data of table 5 are shown graphically in figure 3. In this diagram there has been added the curve of mean monthly production of the Maine Station Barred Rock stock in earlier years, as given by Pearl and Surface (29, p. 89). This shows clearly enough that the laying of the birds in the experiment as a whole has been excellent.

From these data the following points appear to be clearly established:

1. The egg production of the treated birds and the untreated controls was entirely normal in respect of its seasonal distribution, as well as in regard to its amount.

2. There has been no significant difference in the egg production of the treated birds and their untreated control sisters, either in the total average number of eggs produced per bird, nor in the seasonal distribution of this production. Taking the whole untreated flock, the mean production per bird in the 15 months was 184.74 eggs, while the mean production for the treated birds was 183.97, making a difference of 0.77 egg in favor of the untreated. Taking the 'Special Control' mean of 180.80 eggs there is a difference between this and the treated of 3.17 eggs in favor of the treated. Obviously the only conclusion which can be drawn from these insignificant differences is that the inhalation treatment has not affected the egg production of the birds, either favorably or adversely.

3. During the months of July, 1915, to October, 1915, inclusive the mean production of the treated birds falls below that of their control sisters. The difference between the two curves in this region is no greater than may at any time occur between two similarly managed groups of sisters, according to the writer's experience with egg records. There appears to be no reason to attach any significance to this dip of the treated below the control curve. Taking the whole period covered by the diagram it is clear that the two curves wind about one another, now one, now the other being uppermost, just as curves for two random samples of the same material would be expected to do.

V. SUMMARY

A summary of the numerical data regarding the effect of the treatment upon the treated birds themselves is given in table 6. In this table the superior result is printed in bold faced type. In the last column of each table a plus sign denotes that, with reference to the particular character discussed, the alco-

TABLE 6

Showing in summary form the effect of continued administration of alcohol (ethyl and methyl) and ether, by the inhalation method, upon the treated individuals themselves.

CHARACTER OR QUALITY STUDIED	TREATED INDIVIDUALS	UNTREATED CONTROLS	NET RESULT ON ALCOHOLISTS
1. Mean number, per bird of consecutive days of treatment	344.2	0	
2. Net percentage mortality (to February 1, 1916) exclusive of birds accidentally killed	0	41.0	+
3. Mean body weight of females (in gms.)	3266	2953	−
4. Mean egg production per bird, 14 months	183.97	180.80	0
5. General activity	Reduced	Normal	−
6. Sexual activity	Reduced	Normal	−

holists[6] have been favorably affected; a minus sign that they have been unfavorably affected as compared with untreated controls. A zero indicates that no effect of the treatment, one way or the other, has been detected.

From these summarized data it is possible to gain a tolerably clear comprehension of the objective happenings in these experiments so far. The treated animals themselves are not conspicuously worse or better than their untreated control sisters or brothers. The survivors, i.e., those not killed by accident, after roughly a year and a half[7] of daily treatment, are becoming a bit too fat for their best physiological economy, but except for that point, and the reduced activity which goes with it, they are very much like normal fowls. Their apparently much better mortality record is indeed conspicuous, but in view of the small numbers involved, no great significance can be attached to it at present. It is probable that as the experiments proceed this superiority in relative mortality will be considerably diminished. However, as has already been pointed out, the effect of

[6] I adopt this convenient noun from Pearson to denote individuals subjected to the influence of alcohol.

[7] It should be noted that the mean of item 1 in Table 6 is greatly reduced by the fact that all birds, including the methyls and ethers killed in the tanks, are used in its calculation.

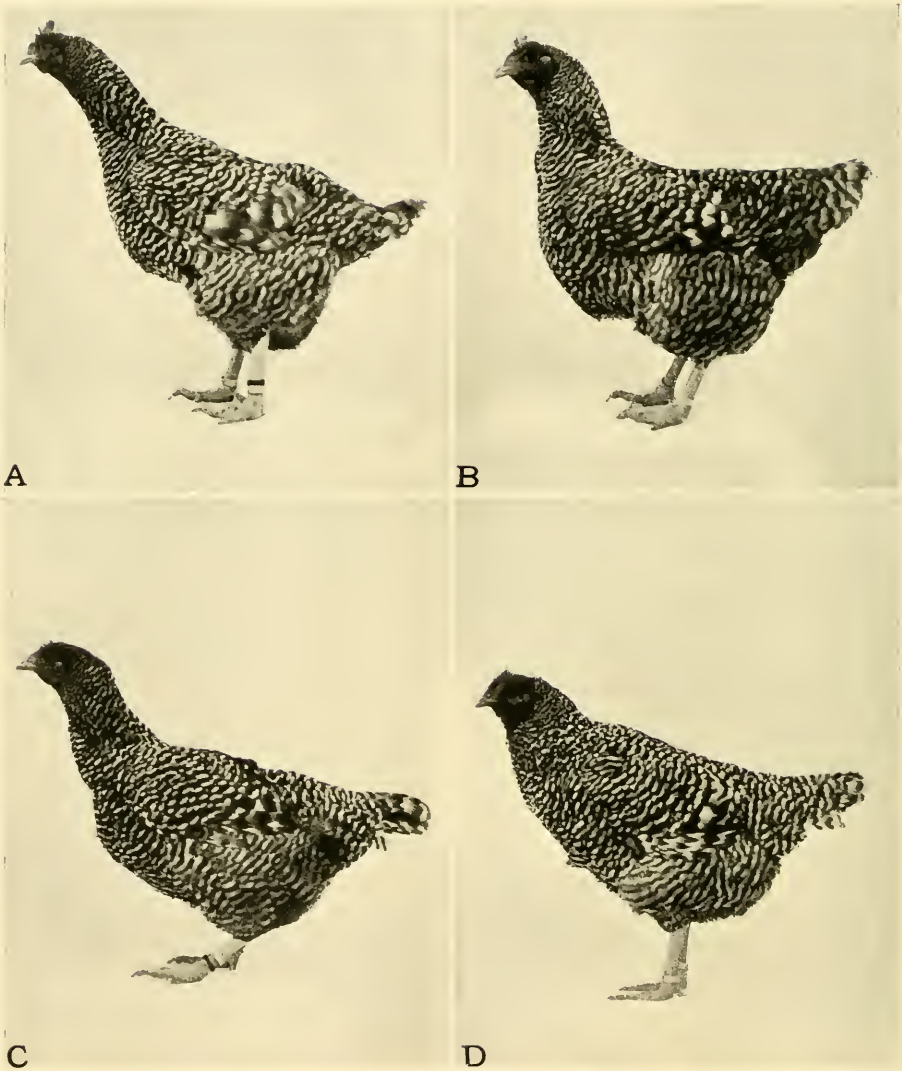

Fig. 4 Photographs of alcoholized Barred Plymouth Rock hens and their untreated control sisters. *A*, ethyl treated ♀ No. 1481; *B*, untreated control ♀ No. 1726, sister of 1481; *C*, ethyl treated ♀ No. 1489; *D*, untreated control ♀ No. 1738, sister of 1489. Scale of reduction in the negative the same for all. Photographed February 18, 1916.

chronic alcoholization upon the duration of life has by no means been well established. There is a widely prevailing popular opinion that even the very moderate use of alcohol shortens life. As is pointed out in the body of this paper, there seems to be no critical evidence as yet that such is in fact the case. The data to this effect which are usually cited are found upon examination not to be critical. As experimental investigations like the present one, and Stockard's with guinea pigs, go on, some rather definite and critical evidence should accumulate regarding this point.

It seems desirable to show by actual photographs that after some 15 months of daily alcohol treatment there is very little visible difference between the treated birds and their untreated sisters. To this end figure 4 has been prepared. This shows two pairs of treated and untreated sisters. *A* and *C* are ethyl treated birds 1481 and 1489; *B* and *D* are their untreated sisters 1726 and 1738. All pictures are to the same scale and were taken the same day, February 18, 1916. It is evident that the alcoholics are in no wise essentially different in appearance from the untreated. The pose of *D* (Bird No. 1738) is bad; she is really just as sprighly and active a bird as any of the others.

Summarily stated the essential results of this paper are:

1 The mortality among the treated birds was much smaller than among their untreated control sisters After 15 months of treatment the difference was 41 per cent in favor of the treated birds.

2. The body weight changes in the treated birds were as follows: immediately following the starting of treatment, which was in the autumn, there was an increase in mean body weight, probably in no way due to the treatment. Following this initial rise, which reached its peak in January or February, there was a sharp and prolonged fall in mean body weight which reached its lowest point in May or June. Beginning in June or July there was a steady increase in mean body weight continuing without break until the end of the period covered in this report (February 1, 1916). At the date mentioned the treated birds

were on the average 9.9 per cent heavier than their untreated sisters.

3. Neither the total amount nor the distribution of egg production were significantly different in the treated birds from what they were in the controls. Both treated and control birds laid normally and well.

A REEXAMINATION OF THE APPLICABILITY OF THE BUNSEN-ROSCOE LAW TO THE PHENOMENA OF ANIMAL HELIOTROPISM

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It has been shown by a number of botanists that the heliotropic reactions of plants obey the Bunsen-Roscoe law whereby the heliotropic effect is determined by the product of the intensity into the duration of illumination. The reactions of free swimming animals to light are generally too quick to permit an examination of the validity of this law, but Ewald[1] has shown that if the efficiency of intermittent and constant light is compared in such forms it is found that both have equal efficiency when the product of duration into intensity of illumination is equal in both cases (Talbot's law.) This proof is in reality also a proof of the fact that the heliotropic reaction is determined by the product of intensity into duration of illumination. It should also be mentioned that the striking results of Bradley Patten[2] on the direction of movements of the negatively heliotropic larvae of the blowfly under the influence of two lights of unequal intensity strongly suggest the validity of some such law as that of Bunsen and Roscoe for heliotropic reactions.

It is, however, desirable to have a direct proof for the applicability of this law to heliotropic reactions of animals. For such a proof we are compelled to turn to sessile animals. Loeb and Ewald[3] have made some preliminary experiments on the hydroid Eudendrium which is positively heliotropic and their observations agreed with the Bunsen and Roscoe law. The number of

[1] Ewald, W. F., Science, 1913, *38*, 236.

[2] Patten, B., Am. Jour. Physiol., 1915, *38*, 313.

[3] Loeb, J., and Ewald, W. F., Zentralbl. f. Physiol., 1914, *27*, 1165.

observations was limited and it seemed desirable to continue these experiments, since it is of fundamental importance to know whether or not apparently purposeful instinctive reactions such as the tropisms can be expressed in terms of purely physico-chemical laws.

The method followed in the work of Loeb and Ewald consisted in ascertaining the time required to cause 50 per cent of the polyps of Eudendrium to bend towards the light, and it was found that this time varied inversely with the square of the distance of the light from the animals. We found that this method could not be followed with satisfaction on account of the great variation in the quality of the material from day to day.

We therefore selected another method. We confined our experiments to three intensities of light by putting the specimens at distances of 25, 37.5, and 50 cm. from a Mazda incandescent lamp of about 33 Hefner candles. The times of exposures were adjusted so that on the assumption of the applicability of the Bunsen-Roscoe law the same effect, i.e., the same percentage of polyps bending towards the light should be produced. Thus in some experiments the exposure for the three distances given was 10, 22.5 and 40 minutes respectively, in others, 7, 15.75, and 28 minutes, and so on. The ratios of the percentage of polyps bending towards the light for the three distances should be as 1 : 1 : 1. Since the material differed widely in different experiments and in different dishes, it was necessary to compute the averages of a large number of experiments.

The source of light was, as stated, a Mazda incandescent electric light of about 33 Hefner candles. This was screened with a series of black screens having circular openings of about 7. 5 cm. in diameter to prevent reflection of stray light.

The colonies, immersed in sea water, were arranged in a row in rectangular glass dishes, the stems being inserted in holes made in a layer of paraffin mixed with lamp black as in the previous experiments. The rear side of the dish was also coated with the paraffin lamp black mixture in order to prevent reflection of light from the slightly uneven back surface of the dish.

The previous treatment of the colonies was similar to that used in the experiments of Loeb and Ewald. After cutting off the existing polyps the stems were allowed to lie for about twenty-eight hours exposed to diffused light. They were then placed in the dark for from eighteen to twenty hours, by which time the hydranths had usually regenerated. The exposure was then made as soon as possible.

In all 3873 hydranths were used or an average of 82 to a dish. Of these 1671 were available for the determinations, i.e., they bent to the light as a result of a short or prolonged exposure. The remaining 2202 hydranths were not available for the determinations, being either refractory or being originally placed parallel to the direction of the rays of light, facing either to the back or front.

At the beginning of an experiment the hydranths bent towards the light were counted, as well as the total number present in the dish. After the exposure the dishes were allowed to remain in the dark for from two to three hours, during which time the heliotropic bending occurred.[4] They were then replaced in light in the same relative position as during the exposure and the polyps turned or bent towards the light were counted. The difference between this number and the number originally turned to the front gave the number of hydranths caused to bend by the exposure. The dish was then allowed to remain in the light for from two to three hours longer and the polyps turned to the light at the end of this period were once more counted. This value less the number turned to the front before the original exposure was taken as 100 per cent in computing the percentage which had been caused to bend by the initial exposure.

The following example will indicate the method of calculation and also prove the fact that the number of polys which bend to the light increases with the duration of exposure.

[4] The very fact that the bending occurred in the dark and not while the organisms were exposed to the light should in itself suffice to prove the untenability of the anthropomorphic explanations of heliotropic reactions by "trial and error" or by hypothetical sensations of brightness.

33 Hefner candle lamp. 50 cm. distance. July 15, 1916

20 minutes exposure.

Number of total hydranths = 58
At start bent backwards = 8
At start bent forwards = 8 } = 16

Hence 42 were apparently available for the experiment. At the end (after 20 minutes exposure and 2 hours in dark) bent forwards 13, i.e. 13 − 8 = 5 actually bent to light; hence $\frac{5}{42}$ = 11.9 per cent reacted of apparently available number of polyps.

After long exposure[5] to same light, 41 bent forwards, showing that 33 were actually available for experiment.

Hence in 20 minutes $\frac{5}{33}$ = 15.1 per cent bent to light.

40 minutes exposure.

Total number of hydranths = 87
At start bent forwards = 15
At start bent backwards = 13 } = 28

Hence 59 were apparently available. At the end of experiment, bent forward 38; hence 38 − 15 = 23 bent under influence of light, i.e., $\frac{23}{59}$ = 39 per cent of apparently available.

After long exposure to same light, 64 bent forward. 64 − 15 = 49 actually available.

Hence in 40 minutes $\frac{23}{49}$ = 47 per cent bent to light.

Fifty minutes exposure gave the same result.

This shows that with the time of exposure the percentage of bending polyps increases until finally all the available polyps bend. The example chosen also shows that it is sometimes impossible to obtain 50 per cent of polyps to bend. The variation in the condition of material makes a large number of experiments necessary.

In our experiments we exposed in the majority of cases only 28 minutes at a distance of 50 cm., in order to avoid the possibility that all the available polyps underwent bending; since in this case the observed ratio of 1 : 1 : 1 for the three distances would have been meaningless. In all the experiments the number of polyps which bent was always smaller, and generally considerably smaller, than the number of available polyps.

The following table gives a summary of the results. The first three columns give the times of exposure for the three distances of the source of light, selected, as stated, on the assumption that

[5] Usually from two to three hours.

TABLE 1

TIMES OF EXPOSURE IN MINUTES			RATIO OF PER CENT OF HYDRANTHS BENDING TOWARDS LIGHT		
25 cm.	37.5 cm.	50 cm.	25 cm. : 37.5 cm.	25 cm. : 50 cm.	37.5 cm. : 50 cm.
15		60		1.50	
20		80		1.30	
10	22.5	40	1.20	(3.08)	(2.56)
10	22.5	40	0.94	1.47	1.55
10	22.5	40	1.57	(2.30)	(2.43)
10	22.5	40	1.43	1.04	0.94
10	22.5	40	0.76	1.09	1.47
10	22.5	40	1.05	1.13	0.90
					0.96
10	22.5	40	1.15		0.99
7	15.75	28	0.84	0.62	0.74
7	15.75	28	1.70	0.49	0.58
7	15.75	28	0.85	1.25	1.35
7	15.75	28	(2.09)[1]	0.99	1.08
7	15.75	28	1.14	1.15	0.55
7	15.75	28	0.44	0.92	0.44
7	15.75	28	1.52	0.80	0.61
7	15.75	28	0.59	0.36	0.70
7	15.75	28	0.48	1.07	0.31
7	15.75	28	1.00	0.48	1.80
7	15.75	28	0.69	1.09	0.81
7	15.75	28	1.26	0.85	1.09
7	15.75	28	0.86	1.38	0.85
7	15.75	28	0.70	1.07	1.59
7	15.75	28		0.77	1.24
7	15.75	28		0.60	
Mean			1.02	0.99	1.02
Probable error			±0.01	±0.01	±0.01

[1] Bracketed values being extreme variates are excluded from calculations of the means and probable errors.

the Bunson-Roscoe law holds. On that assumption the ratio of percentage bent in any two or all three dishes on any one day should equal 1.0. These ratios for each pair of distances of the source of light are given in the three other columns of the table. The percentage bending was only compared in dishes containing material regenerated and exposed on any one day, since only

in this case was there any likelihood that the material was in any way uniform and since only in this case the experiments were carried on at the same temperature and the same conditions of regeneration.

The result was that the observed ratios were as 1.02 : 0.99 : 1.02 (with a probable error of ± 0.01) while the values calculated on the assumption of the validity of the Bunsen-Roscoe law were as 1 : 1 : 1; i.e., the results showed as great an approximation between observed and calculated values as one could expect.

These experiments carried on by a somewhat different method from those previously published by Loeb and Ewald harmonize with the idea that the Bunsen-Roscoe law is the correct expression of the influence of light upon the heliotropic reactions of Eudendrium.

CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOÖLOGY AT HARVARD COLLEGE. NO. 290.

ACTINIAN BEHAVIOR

G. H. PARKER

1. INTRODUCTION

The behavior of actinians has been interpreted in the past in many different ways and the subject even now is open to the greatest uncertainty. Gosse ('60, p. 81), one of the most enthusiastic and industrious students of these animals, after watching the creeping of Sagartia pallida, wrote that "it was impossible to witness the methodical regularity of the process, and the fitness of the mode for attaining the end, without being assured of the existence of both consciousness and will in this low animal form." But such naturalists as Gosse had been schooled to regard adaptations as necessary evidence of intelligence and it was only gradually that these workers were brought to see in Darwin's natural selection one means at least of explaining adaptations without recourse to such a factor. So far has this mechanistic movement gone in the explanation of animal reactions and so vigorously have such workers as Loeb ('99) applied its principles that Baglioni ('13) in his general account of the activities of actinians felt called upon to argue at length for the presence even of nervous action in these forms. It is not my purpose to discuss the question of 'consciousness and will' of the existence of which in these lowly creatures Gosse was so firmly convinced. The futility of such a procedure is too evident. But it is planned to examine some of the more complex activities of these forms with the view of gaining a clearer insight into their elements and into the relation of these elements to the animal as a whole.

The activities of almost every species of organism are directed now into one, now into another of three principal channels; these are, first, the great array of protective measures against unfa-

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vorable features in the environment, next, the maintenance of a normal metabolism, and, last, reproduction. With this final category we shall have nothing in particular to do; under the second we shall take up the matter of feeding, and under the first that of general retraction and expansion.

The elements that are combined in most of these processes have been discussed elsewhere. Actinians such as Metridium possess at least four systems of effectors; slime glands, cilia, nematocysts, and muscles, of which only the last gives evidence of being under nervous influence, and even among these certain muscles are very probably independent effectors. By means of combinations of these elements, the various acts in the appropriation of food, and in retraction and expansion are accomplished. These general activities will be considered on the following pages in the order named.

2. APPROPRIATION OF FOOD

The appropriation of food is an activity with which the oral disc of actinians is principally concerned. The movements of the tentacles, mouth, and other such parts by which food is ingested were ascribed by Nagel ('92, '94) to muscular action alone, but Loeb ('95) pointed out that cilia also play an important rôle. The parts that are immediately concerned in the appropriation of food are the five following: the tentacular gland cells, whose secretions render the tentacles adhesive whereby pieces of food become attached to them; the musculature of the tentacles, by which these organs are pointed toward the mouth; the tentacular cilia, which sweep toward the ends of the tentacles and thus deliver the food to the mouth when the tentacles are pointed in that direction; the transverse muscles of the complete mesenteries, by which the esophagus is opened; and the cilia of the lips and esophagus, which in the presence of food reverse their usual outward stroke and thus transport such materials to the gastro-vascular cavity. Beside these five sets of parts some actinians include in the means by which they appropriate their food a sixth system, namely, the musculature of the oral disc. In Stoichactis, for instance, as described by

Jennings ('05, p. 449) and in Cribrina as reported on by Gee ('13, p. 314), the mouth during feeding is moved by the oral musculature toward the food-bearing tentacles, a shifting which has also been observed in certain corals (Carpenter, '10). This operation, though it can be seen to occur in Metridium, is relatively so insignificant in this form that it may be passed over without comment; the important elements in the feeding of this actinian are the five already mentioned.

Much confusion and uncertainty exists in the various accounts of the methods by which actinians obtain their food and more or less of this is due to the failure on the part of writers to designate the particular form of activity that they are for the moment discussing. Thus both ciliary and muscular activity are involved in the appropriation of food and have often been indiscriminately dealt with in accounts of this operation. Their significance for the animal as a whole is, however, very different and it is, therefore, highly desirable that they should be kept clearly in mind as separate processes in any discussion in which they are involved.

Of the five principal events that go to make up the act of food appropriation, three exhibit so little variation that they may be regarded as essentially uniform. These are the secretion of mucus, the beat of the tentacular cilia, and the opening of the esophagus. In none of these are there during feeding any important readjustments which are essential to the acquisition of food; the production of mucus is apparently a strictly local response to a local stimulus; the beat of the tentacular cilia is constant and irreversible; and the opening of the esophagus is as simple and mechanical a reflex as could well be imagined. The idea that the esophagus, as often intimated, exhibits peristalsis is probably incorrect. At least a careful inspection of this organ in action in Metridium gives no support to this idea. The two remaining events in the appropriation of food, the responses of the oral cilia and the movements of the tentacles, are both open to significant changes and are of the utmost importance in judging of the relation of this process to the actinian as a whole.

Unlike the tentacular cilia, the oral cilia, those of the lips and the esophagus, may reverse the direction of their stroke so that the usual outward current can be converted into an inward one. This reversal is under ordinary circumstances a local response on the part of the cilia to certain dissolved substances in the food. Its relative independence of the other activities of Metridium can be shown in a number of ways. Thus, though it is a response to food, excessive feeding has no marked influence on it. Allabach ('05, p. 38) caused a Metridium to gorge itself with food, a process which can result finally in disgorgement, and yet immediately after the animal had emptied itself, its oral cilia were found to reverse to food, which was thus passed down its esophagus. My own observations confirm this statement. Further if pieces of meat are fed to the lips of the oral half of a Metridium cut transversely in two, the cilia reverse and the masses of food thus carried through the esophagus are discharged at its open pedal end. By this means in the course of an hour or so I have passed through the esophagus of a Metridium many times the amount of food that its body could have contained, and yet the ciliary reversal was as effective after this period of continuous feeding as before.

Other evidence of the relative independence of the oral cilia as compared with other effectors is well seen in specimens of Metridium that have been narcotized with chloretone, by which all nervous activity is abolished. A piece of food placed upon the tentacles of such an animal calls forth no special response and either remains where it was placed or moves sluggishly off to the periphery of the disc under the action of the tentacular cilia. When, however, such a piece is put on the lips, the cilia reverse and the morsel is gradually carried down the esophagus and discharged into the gastrovascular cavity. The swallowing is usually not so rapid as in the normal animal for, under this form of narcotization, the transverse muscles of the mesenteries do not respond to the food by opening the esophagus and consequently the cilia are obliged not only to transport the morsel but to force it down a partly closed tube. This, however, they are usually able to do and thus quite independent of neuro-

muscular help, they bring about the swallowing of food and the rejection of non-food, for under these circumstances inert materials were found not to reverse the ciliary stroke. Thus, as Allabach ('05, p. 38) has pointed out, the reversal of the effective stroke of the oral cilia is a process which is largely independent of the physiological state of Metridium.

In one particular only does this process appear to be related to the general condition of the animal. Ordinarily the reversal of the oral cilia is accomplished by dissolved substances from the food and in my earlier studies on Metridium I was able to get this reversal only by such means. Torrey ('04), however, showed that in Sagartia this reversal could be brought about by mechanical stimuli as well as by chemical means and that it was favored by a starved condition of the animal. Allabach ('05) also found that in Metridium a ciliary reversal could be induced by mechanical means and Gee ('13) has recently shown that specimens of Cribrina which have been in the laboratory some time do not exhibit a reversal to mechanical stimuli, whereas those still in their native pools give evidence of it.

From my own reinvestigation of the question, I am led to agree with Allabach ('05, p. 37) that in Metridium marginatum some individuals on mechanical stimulation reverse their ciliary stroke readily, others less readily, and still others not at all, variations largely dependent upon whether the animals have been starved or fed. Two underfed specimens of Metridium which on being tested were found to reverse their cilia to clean filter-paper were vigorously overfed and after three hours were tested again with bits of clean filter-paper. In both instances the paper failed to bring about a reversal of the cilia and consequently was ejected. In another test made eighteen hours after feeding, the paper was engulfed showing that the cilia had returned to the state characteristic of animals that had lacked food. I therefore, believe, contrary to my former opinion, that an underfed Metridium will reverse the effective stroke of its oral cilia to mechanical stimulation, though a small supply of food will obliterate this peculiarity and leave these organs incapable of such reversal.

The occasion of this loss of the power to reverse the stroke of the oral cilia on mechanical stimulation has been ascribed by Allabach ('05, p. 39) to the difference in metabolism between a well fed and an underfed individual. I have tested this by cutting out the esophageal tubes from several specimens of Metridium, laying them open and experimenting with them as ciliated membranes. If they are carefully prepared from animals that have not been recently fed, they will show a well marked ciliary reversal to pieces of clean filter-paper. To fragments of mussel they reverse the ciliary stroke in the way characteristic for food and after a dozen or more such trials they will no longer reverse to pieces of clean filter-paper. Thus the isolated membrane exhibits all the changes that it does as a part of the whole animal and under conditions where it is quite obvious that the one change that it has suffered is fatigue. I therefore believe that the general metabolism of Metridium is not so much concerned with the change in the character of the response of the cilia to filter-paper as the fatiguing of the receptive mechanism of the ciliated surface is. In the undisturbed state this mechanism is at its greatest sensitiveness but on feeding its efficiency diminishes and hence filter-paper no longer excites a reversal, a change which is now called forth only by the more vigorous stimulation from the dissolved products of the food. Hence in my opinion the activities of the oral cilia are more independent of the rest of the actinian than even Allabach ('05, p. 38) was inclined to insist upon.

The feeding movements of the tentacles in actinians are obvious neuromuscular reactions, as their disappearance on narcotization with chloretone amply shows. The independence of the individual tentacles in their feeding reactions has been demonstrated in a number of forms, in which these responses have been observed after the tentacles have been cut from the polyp. That one tentacle can influence another through connections in the oral disc has been proved for Condylactis and is probably true for Metridium. The muscular responses of the tentacles in feeding, therefore, give much more opportunity for unified action than do the ciliary responses just considered.

That tentacular responses in actinians change with continued activity has long been recognized. Jennings ('05, p. 400) found that the tentacles of Stoichactis after they had been vigorously plied for a while with meat ceased for a time to react to food. Allabach ('05, p. 38) noted that in Metridium the tentacular reactions became gradually slower or even ceased as feeding progressed, and the same is recorded by Gee ('13, p. 320) for Cribrina. I long ago published evidence of this in Metridium and my recent work on this point is entirely confirmatory.

Jennings ('05) attempted to explain this change as due to loss of hunger,[1] but (Allabach, '05) showed that it also occurred when the tentacles were stimulated but the animal was not allowed to swallow the food. Her conclusion is that it is simply the effect of fatigue. Gee ('13, p. 324), however, declined to accept this explanation because if an actinian that will ordinarily show this tentacular change after having been fed eight or ten times, is experimented upon when in a fresh condition and is made to contract about the same number of times, its tentacles are found not to have lost their responsiveness. But both Allabach and Gee have failed to recognize that there are several kinds of fatigue. It is perfectly clear, from Gee's experiment, that muscular fatigue is not accountable for the change in the responsiveness of the tentacles, but it is entirely possible that it may have been caused by sensory fatigue. It is a common observation that if a sensory surface is placed under active stimulation, it is often only a short time before it will fall off very considerably in its receptiveness and it is this form of fatigue, I believe, that is accountable for the change in the tentacular responses of Metridium on continuous feeding. I have had occasion several times to repeat Allabach's experiment of placing food on the tentacles of Metridium and, after they have responded, of removing it from the lips before it was swallowed, and in all instances

[1] It is perhaps unfortunate that the term hunger should have been used, for it is somewhat ambiguous. Usually it stands for a well known sensation due to movements of the stomach (Cannon and Washburn, '12); less commonly for insufficient bodily nutrition. Pathology has long since demonstrated that these two phenomena are not necessarily connected, but in which sense Jennings intended to use the term is not always wholly clear.

I can confirm her results, namely, the tentacles fall off in responsiveness. In view of what has already been stated I am unable to explain this phenomenon except as a result of sensory fatigue.

But there are also changes in the tentacular responses of actinians that are by no means so easily explained as are those that have just been considered. Jennings ('05, p. 457) states that when the tentacles on the left side of an Aiptasia were plied with crab meat, they transferred the food to the mouth quickly five times, after which they reacted slowly on the sixth trial and hardly at all on the seventh.

On trying the meat on the tentacles of the right side, it was found that the transfer to the mouth was quickly accomplished. Returning now to the left side four sluggish deliveries were effected after which the right side would now take no meat at all. Allabach ('05, p. 39) states that Metridium can be fed from one side of its disc till no more food will be accepted, whereupon food will likewise not be accepted by the tentacles of the opposite side. Gee ('13, p. 321) has also recorded essentially the same condition in Cribrina. From these observations it seems clear that changes induced in the muscular responses of the tentacles of one side profoundly influence the reactions of the tentacles on the other side. As Jennings ('05, p. 457) has put it, the animal reacts as a unit, one side influencing the other.

I have repeated experiments of this kind on Metridium and though my results are not as striking as those described by the authors already quoted, I am convinced that when a Metridium is fed persistently by means of the tentacles of one side and so as to avoid touching with the food those of the other side, the opposite tentacles are nevertheless eventually influenced in their reactiveness and become less responsive as the feeding proceeds. Here would seem to be a good instance of some such general effect as that of changed metabolism or the general utilization even of nervous experience.

To ascertain whether changes in the tentacular responses of one side of the disc are transmitted nervously to the other side, I fed small pieces of mussel to the tentacles of one side of a

Metridium but removed them before they were swallowed and then, after the tentacles of that side began to lose in responsiveness, I tested those of the other side to see if they too had lost in their capacity to respond. The times in seconds required for the swallowing of each piece of food are recorded in the following table. The rejection of a piece of food is indicated by the sign of infinity.

It must be evident from an inspection of table I that the right side of the animal gave no evidence of having been influenced by the left and that therefore we are not warranted in

TABLE I

Time in seconds for the transfer by the tentacles of Metridium of small pieces of mussel to the mouth whereupon they were removed as they were about to be swallowed. Sixteen trials were made on the left side and then the same number on the right. ∞ indicates a discharge of the piece of meat at the periphery of the oral disc

	NUMBER OF THE TRIAL															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Left side of disc.....	284	121	107	86	103	92	71	58	∞	97	108	∞	∞	72	∞	∞
Right side of disc.....	72	86	306	63	112	∞	83	132	74	96	103	97	86	62	78	109

assuming that the experience of one side is transmitted nervously to the other. In other experiments, in which the fragments of mussel delivered to the tentacles of the first side were allowed to be swallowed instead of being removed, the tentacles of the opposite side very regularly exhibited a decline in responsiveness. I therefore believe that this change is due to the food introduced into the gastrovascular cavity, and, since the pieces of food were very small, not to the accidental transfer of food juices from the side of the disc stimulated to the other. as suggested by Gee ('13).

To remove any doubt on this point I adopted a modification of an experiment tried by Gee ('13) and injected by means of a fine glass syringe through the column wall of small specimens of Metridium a considerable amount of mussel juice into their gastrovascular spaces. This operation is easily accomplished

especially if the region through which the puncture is made is previously anesthetised with magnesium sulphate. I could not see that the injected juice escaped from the mouths of the animals which, however, took in a considerable amount of sea-water and enlarged much as well fed actinians do. After an hour or so I tested the tentacles of the injected actinians with fragments of mussel and found them very noticeably insensitive to food. It therefore seemed clear that it was the food in the gastrovascular cavity rather than any accidental overflow that had influenced the tentacles.

I have already pointed out reasons for believing that the change in the responses of the tentacles after continuous feeding is due to sensory fatigue and not to a general metabolic change and I believe that the same is also true in the particular instance under consideration. Though the meat juice injected into the gastrovascular cavity unquestionably serves as material for metabolism and eventually must have its influence on the animal's general state, its first condition is that of a component of the fluid mixture which bathes the inner surfaces of the actinian. These surfaces include the cavities of the tentacles. As I have shown elsewhere (Parker, '17a) substances in solution in the gastrovascular space of such organs as the large tentacles of Condylactis penetrate in a very short time the thin walls of these parts and thus make their way to the exterior. In doing so they must come in contact with the sensory ectoderm. Since the changes in the reactions of the tentacles produced by food juices injected into the gastrovascular cavity are in the direction of diminished response and since these changes come over the tentacles with considerable rapidity and before a modified metabolism dependent upon new food could have got much headway, I believe that the loss of responsiveness in this instance, like that in the former case, is due to sensory fatigue and not changed metabolism. In the first instance the fatigue was produced by the direct application of stimulating substances to the exterior of the tentacles; in the second to the transfusion of those substances from the cavities of the tentacles to their sensory mechanism. If this explanation is correct, as there is good reason

to suppose it is, the responses of the tentacles are like those of the oral cilia in that they are not especially dependent upon the condition of the animal as a whole.

As Gee ('13, p. 326) states, "the view that the seat of the modified responsiveness lies very largely in the individual tentacles is more clearly in accord with what is known of the structural organization of the sea-anemone than that the animal acts as a unit."

The appropriation of food by sea-anemones then is a process which involves factors none of which necessitate the assumption of the action of the animal as a whole. All are most strikingly local and the changes that they exhibit are apparently entirely due to fatigue. In these respects they are in strong contrast with food appropriation in the higher animals, a process which has become so deeply wrought into the make-up of these forms that its relation to the animal as a whole is most profound. While almost every one of the elements involved in actinian food appropriation may be experimentally isolated and made to act for itself in a most remarkably local way, scarcely any such independence is observable in the parts concerned in the similar operations of higher animals; the jaws and their muscles, buccal glands and so forth in these higher animals exhibit a highly unified action dependent chiefly upon central nervous connections such as is scarcely suggested in actinians, but as isolated elements they have almost no reactive power at all as compared with what is possible in sea-anemones. Food appropriation in actinians then emphasizes rather the relative independence of parts than the action of the organism as a whole.

3. RETRACTION AND EXPANSION

As the locomotor activities of Metridium, and in truth of most other actinians, are extremely limited, the chief protective response of these animals is general retraction whereby they are reduced greatly in bulk, their more delicate parts are brought under cover, and they shrink close to the substratum to which they are attached. In many instances in fact retraction brings about a withdrawal of the body of the actinian into deep, rocky

recesses and the like whereby very efficient protection is secured. The reverse process, expansion, is one which involves an enlargement and protrusion of the body as a whole and the opening of its folded surfaces and apertures in such a way that the operations of feeding, respiration, and so forth may be resumed.

The means by which retraction and expansion are carried out have already been partly described (Parker, '16). Retraction in its initial phases is chiefly the result of the action of the mesenteric muscles, the longitudinal muscles of the non-directive mesenteries depressing the oral disc, those of the directives serving chiefly to fold the siphonoglyphs, and the parietal muscles acting on the column wall. After the depression of the oral disc has proceeded somewhat, the contraction of the sphincter muscle completes the process by bringing the oral disc under cover through the puckering effect of this muscle on the column wall. Incidentally the process of general retraction involves the expulsion of almost all the water contained in the gastrovascular cavity of the actinian. The reverse operation, expansion, is dependent first of all upon the relaxation of the sphincter and of the mesenteric muscles; then follows the slow filling of the gastrovascular spaces with sea-water through the ciliary currents in the siphonoglyphs; and probably as a last step the circular muscles of the column contract on the fluid contents of the body whereby the oral disc is forced well up above the pedal attachment. The details involved in the processes of retraction and expansion allow retraction to be accomplished much more quickly than expansion. This relation has all the appearance of an adaptation, for the quickness of a withdrawal may often be the essential part of the protection given by retraction, whereas there is nothing about the economy of an actinian, such as feeding, respiration, and so forth, that makes it vitally important for the animal to expand quickly.

The conditions under which a Metridium remains fully expanded are by no means simple but include an aggregate of factors. In the laboratory the fullest expansion was obtained when the animals were in well-oxygenated, cool, running sea-water in the dark. Under such circumstances this sea-anemone

will extend itself to as much as six times the diameter of its column, and hold its oral disc fully opened. In no instance have I ever found in nature a degree of expansion greater than that seen in the laboratory under the circumstances just stated. This maximum degree of expansion under natural circumstances has often been observed in sea-anemones in pools during the night or even during the day in dark situations such as under bridges and so forth. The elements that contribute to this extreme expansion are certainly diverse. Of these I have tested light, temperature, food, oxygen supply, and water currents.

The influence of light on actinians is by no means uniform but differs with different species. According to Nagel ('94, p. 545; '96, p. 33) Adamsia, Anemonia[2] and Actinia are not responsive to light. Fleure and Walton ('07, p. 217) have noted this lack of response in Anthea as well as in Adamsia. Piéron ('06 c, p. 44; '08 c, p. 1021) has confirmed Nagel's statement for Actinia. Although this lack of response may be true of the forms just mentioned, I have not been able to demonstrate it in Metridium marginatum nor in Sagartia luciae, both of which according to Hargitt ('07, p. 280) are said to be quite indifferent to light. My observations on these species leave no doubt in my own mind that both close quickly on bright illumination. This is in agreement with Bohn's observations ('06 a, p. 421) as well as with Gosse's account ('60, p. 15) of the closely allied species, Metridium dianthus. Concerning this form Gosse remarks that "it is under the veil of night that the anemones in general expand most readily and fully. While the glare of day is upon them, they are often chary of displaying their blossomed beauties; but an hour of darkness will often suffice to overcome the reluctance of the coyest. The species before us," M. dianthus, "is not particularly shy; it may often be seen opened to the full in broad daylight; but if you would make sure of seeing it in all the gorgeousness of its magnificent bloom, visit your tank with a candle an hour or two after nightfall." Retraction under

[2] Bohn ('07 c) states that Anemonia is not entirely without response to light. In weak light it is said to place its tentacles at right angles to the rays and in strong light parallel to them.

bright illumination has also been recorded for a number of actinians among which are the following: Edwardsia[3] (de Quatrefages, '42, p. 76; Fischer, '88, p. 23), Cerianthus[3] (Haime, '54, p. 348; Nagel, '94, p. 545; Hess, '13, p. 438), Phillia (Gosse, '60, p. 350), various species of Sagartia (Gosse, '60, pp. 81, 111; Fleure and Walton, '07, p. 217; Hargitt, '07, p. 275; Piéron, ('08 c, p. 1021), Paractis (Jourdan, '79, p. 28), Cladactis (Hertwig, '79–80, p. 56), Aiptasia (Jennings, '05, p. 459), Tealia (Fleure and Walton, '07, p. 217), Eloactis (Hargitt, '07, p. 275), Ceractis (Schmid, '11, p. 538), and Bunodes (Hess, '13, p. 438). Although closure in the presence of light is the ordinary form of response for most actinians, there seems to be good evidence that a few react in the opposite way. Actinia equina according to Bohn ('08 a) is expanded in the daytime and retracted at night and the same is true of Cribrina zanthogrammica as observed by Gee ('13, p. 309), who also adds that a closed Cribrina in the dark will expand under the influence of a 32 candlepower light. Both Actinia and Cribrina contain symbiotic algae in their tissues and it is easy to imagine that their expansion in daylight may be an advantage so far as photosynthesis is concerned, but whether this expansion is a reversal of the usual form of actinian response to light or is due to the effects of some such substance as oxygen which may be given off by symbiotic alga in the light is not known. It thus appears that aside from a few indifferent actinians and a few that open in the light, the majority respond to the stimulus by retraction. In this respect, as already intimated, Metridium is not exceptional.

If a fully expanded Metridium in the dark is suddenly exposed to diffuse daylight, it will shorten its column to one-third or one-fourth its former length and with its oral disc fully expanded remain in this state more or less continuously. The shortened state produced in Metridium by general illumination represents the ordinary condition in which many of these sea-anemones are found in nature during the daytime. If on such a partly

[3] These instances, Edwardsia and Cerianthus, are often attributed to Bronn ('60, p. 23) who apparently simply repeated the statements made by de Quatrefages and by Haime without giving references.

contracted Metridium a beam of reflected sunlight is thrown, the animal will after a minute or so almost invariably shorten its column completely and contract its oral disc, thus assuming the condition of complete retraction. This state is commonly met with in nature as a result of direct exposure to sunlight. It occurs in situations where the sea-anemones are subjected during a part of the day to shadow and during the rest to full sunlight. Under the latter circumstances they are almost invariably fully retracted; under the former they are more or less expanded.

When a fully expanded Metridium in running water in the dark is illuminated either from the side and from above by a 16 candlepower electric light at a distance of half a meter the animal will shorten considerably but, as a rule, not cover the oral disc. This was occasionally induced by very strong artificial illumination, but it is a reaction by no means easily called forth. It was however often enough met with to warrant the conclusion that so far as light is concerned Metridium will undergo complete contraction of both column and oral disc only in the very brightest illumination; that in weaker light it shortens the column but does not cover the oral disc and that its fullest expansion is called forth only in complete darkness.

The effect of the temperature on actinian response has been little studied. The specimens of Metridium upon which my observations were made were kept in an aquarium with running seawater, the temperature of which was about 23°C. The temperature of the outside water from which the supply for the aquarium was obtained was about 21°C. (August). At such temperatures, as was to be expected, the animals remained expanded when the other conditions were appropriate, and normally responsive. When the animals were supplied with running seawater that had been artificially cooled to about 8°C, they remained fully expanded in the dark and would shorten in the light. They responded to a mechanical stimulus by contraction, and in other respects they reacted as they did under more usual temperatures.

If sea-anemones in seawater at 23°C. are flooded with water at 35°C. even though they are kept in the dark, they invariably contract completely. This response is in agreement with what

was found by Fleure and Walton ('07, p. 217), namely that Actinia and Anthea retract at temperatures above 22°C. If, however, Metridium is subjected to a gradual change of temperature which eventually reached 36°C., it slowly loses its responsiveness to mechanical and chemical stimuli and soon dies. The loss of responsiveness begins at about 34°C., and is complete at 36°C. An animal kept a few minutes in seawater at 35°C. may be touched repeatedly on the column near the pedal disc without showing any response and may be eventually killed in alcohol in an expanded condition. Animals which have thus been rendered insensitive seldom recover but in the course of a day or so die.

So far as Metridium is concerned subnormal temperatures have little influence on its responsiveness except possibly on the rate. Supernormal temperatures, if quickly applied, induce general contraction; if gradually applied and of sufficient intensity (35°C), they bring about a condition of non-responsiveness that quickly passes over without contraction into one of death. I made no attempt to localize the receptors for differences of temperature (if, in fact, this response is dependent upon receptors) and I am, therefore, not in a position to confirm or deny Nagel's statement ('94, p. 337) that the tentacles are the organs concerned.

Many observers in the past have noted that retracted sea-anemones can be induced to expand by placing pieces of meat or other food so near them in the water that dissolved materials from this food are wafted to the animals. Pollock ('83, p. 474) and Romanes, in consequence of such observations, were led to assume the presence of the olfactory sense in these animals. More recently this response to food has been observed in Metridium by Allabach ('05, p. 37) and in Actinia by Piéron ('06 b, '06 c). So far as Metridium is concerned, I can fully confirm Allabach's statement. If into two large glass dishes of fresh seawater many specimens of contracted Metridium are placed and into one of these dishes is poured a small amount of juice from a crushed Mytilus edulis, the sea-anemones in that dish almost without exception will expand their oral discs in a very

few minutes, whereas those in the other dish will remain almost to an individual retracted. It was quite clear to me from observations of this kind that the dissolved products from the food of the sea-anemone would induce the expansion of its oral disc though this agent had very little effect on the shortened condition of the column in these animals.

The part played by oxygen in the expansion and retraction of sea-anemones has been a matter of recent dispute. According to Piéron ('06 b) Actinia equina opens in seawater with a large oxygen content and closes when there is a deficiency of this gas. Piéron ('08 a, '08 b, '09) as a result of further investigations was led to believe that not only did oxygen have this effect but that it was one of the most important factors in determining expansion and retraction. Bohn ('08 a, '08 c, '10 a) on the other hand maintains that Actinia equina will remain expanded in seawater containing very little oxygen and will close when that water is richly oxygenated. In the opinion of this investigator the states of expansion and retraction are due chiefly to light and darkness and not to the supply of oxygen. In the face of such differences of opinion it is difficult to arrive at any conclusion without further observation.

All the experiments that I made to ascertain the importance of oxygen in retraction and expansion were carried out on Metridium marginatum at Woods Hole, Massachusetts. Specimens of this species were studied in rock pools which were flooded at high tide and left isolated at low tide. The oxygen content of the seawater from the several situations involved was determined by the Winckler method. I am under obligations to Dr. H. Wasteneys for having made these determinations for me. The outside water on the incoming tide was found to contain 7.06 mgm. of oxygen per 1000 cc. The water in a small undisturbed pool just previous to the entrance of the tide contained 3.15 mgm. of oxygen per 1000 cc., while that in the undisturbed end of a pool into which the tide was beginning to flow, contained 2.76 mgm. At the end of the pool into which the tide had entered, the oxygen was found to be 7.02 mgm. per 1000 cc. From these figures it is evident that at the time of

observation the water in the pools contained decidedly less than half as much oxygen as that in the flowing tide and that the entrance of the tide into a pool quickly changed the water there from a condition poor in oxygen to one relatively rich in this gas. Another point of difference in the water of the pool and that of the flowing tide was that the pool water had a temperature of about 27.5°C. and that in the inflowing tide 21.5°C.

Experiments to ascertain the effect of the oxygen in the several kinds of water on Metridium were conducted under the following conditions. The pools, which were on Pine Island, in Woods Hole, Massachusetts, were studied on clear days in August. Work was begun as the tide was rising but before it had reached the pools. In bright daylight almost all the specimens of Metridium were retracted. Large battery jars were carefully filled with water from the pools and into these jars stones were put having attached to them several specimens of Metridium in the retracted condition. The jars were allowed to stand in the same exposure as the pool to determine whether the act of transfering the sea-anemones would influence their conditions. As a matter of fact the animals remained closed and gave no evidence of being in any other state than that of the actinians that remained in the pools. Careful transfer from pool to jar is therefore not a source of disturbance to Metridium.

If, now, pieces of stone on which there are closed actinians are quickly transferred from the pools to the outside tidal water, many of the sea-anemones on them will in a few minutes expand their oral discs though their columns will remain contracted. This response, though not invariable, was of such common occurrence that it was quite obviously typical of the transfer. It must depend upon some difference between the two bodies of water, for as has already been shown, the act of transfer in itself is without significance. The difference between the two bodies of water are differences of temperature, oxygen content, and current action.

To ascertain the effect of temperature, carefully collected pool-water was cooled by being surrounded with ice from its initial temperature of 27.5°C to that of the outside tidal water, 21.5°C.

Into this cool pool-water pieces of rock from the pools on which were closed actinians were introduced, and the animals watched. They remained contracted for over an hour and it was concluded that the expansion of the sea-anemones when transferred from the pools to the outside tidal water was not due to the difference in temperature.

Pool-water was now collected and thoroughly aerated by being poured back and forth from one jar to another many times, but when placed in this the sea-anemones also failed to expand. Some of this water on being examined proved to contain 7.33 mgm. of oxygen per 1000 cc. It is therefore clear that Metridium does not expand in the running tide because of the increase of oxygen.

Finally two jars were so arranged that one conducted water into the other through a large siphon in such a way as to expose the flowing water to air as little as possible. The upper jar being kept full of pool-water, supplied the lower jar from which the water was in continuous overflow. In this way pool-water was given a current without changing in any marked degree its temperature or its oxygen content. When closed specimens of Metridium on bits of rock were introduced into the jar through which the water was flowing, they very commonly expanded their oral discs though their columns remained short. I therefore concluded that the motion of the tide water, rather than its lower temperature or greater oxygen content, was the element responsible for the expansion of Metridium under the circumstances noted. As a check on this conclusion several vessels were filled with tidal water and after it had come to rest stones carrying Metridium were introduced into it. Although this quiet tidal water retained its characteristically lower temperature and its higher oxygen content, the sea-anemones remained closed in it, thus confirming the conclusion already expressed that motion is the element in tidal water that induces expansion.

The effect of water currents and other forms of agitation were not only observed under natural conditions but were tested likewise in the laboratory. If a Metridium is put in a darkened vessel through which seawater is running, it quickly assumes

a condition of maximum expansion both as to its oral disc and its column. If, now, the current is shut off, in about a quarter of an hour the oral disc will be found covered but the column will remain more or less elongated. The same was found true of groups of Metridium on stones. Five, in one group, were made to expand fully in running seawater in the dark. The current was then cut off and in eighteen minutes the oral discs of all five specimens were covered and some of the animals a little shortened. An hour and a half after the current had been stopped all were still closed except one which had partly expanded its oral disc. Still an hour later all were retracted, whereupon the current was reestablished and in seven minutes all were expanding, a process completed by all five in about thirteen minutes. These responses were found to occur as well at 8°C. as at the more usual temperature of 21°C.

The agitation of the seawater, in a purely mechanical way and without reference to oxygen and the like, appears, therefore, to be a means of inducing the expansion of Metridium, especially of its oral disc. This form of reaction has already been observed in Actinia by Piéron ('06 b, '08 d), who, however, points out that certain forms of mechanical agitation also induce retraction (Bohn, '07 a).

Since the expansion of the oral disc is dependent chiefly upon the relaxation of the sphincter muscle, it seems probable, as already pointed out (Parker, '16), that the mechanical stimulus of the moving water in one way or another has a very specific effect on this muscle. The condition of relaxation thus induced is apparently exactly like that seen in such sponges as Stylotella, where the oscular sphincter remains relaxed in running water but contracts when the current ceases (Parker, '10).

The foregoing account shows quite clearly that the expansion and retraction of such a sea-anemone as Metridium is dependent upon a variety of factors. Light and high temperature, especially when suddenly applied, produce retraction; food and water currents, expansion; the oxygen supply, in Metridium at least,

seems to have very little if any direct influence on retraction and expansion.

These operations in sea-anemones have been regarded by some investigators, notably Bohn and Piéron, as occurring in rhythmic fashion, and two types of rhythm have been distinguished; a tidal rhythm and a daily or nychthemeral rhythm. According to Bohn ('06 b, '09 b, '10 b) Actinia equina retracts when it is exposed to air by the falling tide and expands when it is again covered by water. This rhythm may be retained for from 3 to 8 days in an aquarium though the animals under such conditions are always under water. Piéron ('08 c) on the contrary questions the presence of a pronounced tidal rhythm in Actinia equina.

Metridium marginatum is found commonly either below low-water or in pools that do not empty on the falling of the tide. When exposed to the air it usually retracts though this is not invariable. This species, partly from the situations in which it is found and partly from its irregularity of response, is not a very favorable one in which to seek evidence of tidal rhythm.

In this respect Sagartia luciae is very much more promising. This species attaches itself to stones, shells, and other fixed objects that are commonly exposed to air by the falling tide. When thus exposed this species is very regularly retracted, and when covered with water it is expanded though not invariably so. To ascertain whether this rhythm would persist, as maintained by Bohn for Actinia, I transferred at various times to an aquarium stones covered with Sagartia luciae and kept records of their subsequent conditions. My results were quite uniform and may be well illustrated by a single example. On July 7 at 11.00 in the morning a stone that had been exposed by the tide for some hours and that had upon it twelve contracted Sagartia was transferred to an aquarium. At half past eleven all the sea-anemones had expanded and they remained so for the next thirty hours, after which they began to close irregularly. Similar conditions were repeatedly observed and I am quite sure that in Sagartia luciae there is no persistence of a tidal rhythm.

In this respect my observations agree with those of Gee ('13, p. 310) on Cribrina, where no trace of the persistence of tidal rhythm could be discovered.

Metridium marginatum is almost always under water and is so responsive to light that it might well be suspected to be a species that would exhibit a pronounced daily or nychthemeral rhythm. On August 9 at 10.30 in the morning a large pool in full sunlight was plotted and twenty large specimens of Metridium were accurately located. All were fully retracted. At 10 o'clock on the evening of the same day, the sky being overcast with clouds and the night dark, the pool was again visited and by means of a hand light the twenty sea-anemones were reidentified. All were fully expanded. A number of other observations of this kind and many casual records were made of the condition of pool animals in daytime and at night, and always with the same results; the sea-anemones were fully expanded at night and partly or completely retracted in the day. Observations on animals located under bridges and in other dark situations showed that they were more or less continuously expanded, but aside from such exceptions it was clear that Metridium in its natural surroundings exhibited a well marked nychthemeral rhythm.

This form of rhythm agrees with what Hargitt ('07) has observed in Eloactis, and Piéron ('08 c) in Sagartia troglodytes, and what has been claimed by Bohn ('06 b, '07 b) to occur in Actinia equina, though the nychthemeral rhythm in this species has been questioned by Piéron ('08 c, '08 e). That in Metridium it is dependent upon light, as maintained in general by Bohn ('08 a, '10 a), and not upon oxygen, as was claimed for other species by Piéron ('08 c, '08 e), has already been shown in an earlier part of this paper.

I have never observed anything about the activities of Metridium that would lead me to suppose that its nychthemeral rhythm is ever reversed or is ever exchanged for a tidal rhythm as has been claimed for some species by Bohn ('08 b, '09 b).

A persistence of the nychthemeral rhythm in Metridium after its removal from the influence of day and night is apparently

as little in evidence as the tidal rhythm in Sagartia luciae. Specimens of Metridium in a retracted condition were removed from a quiet pool at noon and placed in running water in the dark. In less than an hour all were fully expanded and remained so for over 36 hours. These observations agree with those of Gee ('13, p. 310), who was unable to find any evidence for the persistence of the nychthemeral rhythm in Cribrina.

That sea-anemones may exhibit in the sequence of their states of expansion and retraction a tidal rhythm or a nychthemeral rhythm, as pointed out by Bohn and by Piéron, there can be not the least question, but that these rhythms may persist even for a few days in the absence of the external stimulus, as maintained especially by Bohn, is certainly not true for Metridium marginatum nor Sagartia luciae. The fact that a persistence of rhythm in aquarium specimen has not been seen by Appellöf (Retterer, '07), Gee ('13), and others throws great doubt on the occurrence of this phenomenon at all, but a decisive answer to this question can not be given till the species for which these peculiarities have been claimed are reinvestigated.

Bohn and Piéron ('06), and especially Piéron ('06 b, '08 a, '10) have claimed that in Actinia equina the tidal rhythm is carried out a little in advance of the actual tidal changes, thus giving evidence of what may be called an anticipatory reaction. This reaction, according to Piéron ('10), may be lost when the animals are placed in an aquarium and may be regained after a week or so when they are again subjected to the tides. I have watched Metridium very closely for signs of this preparatory activity, but I have never seen any conclusive evidence of it. It is astounding how quickly Metridium will begin to expand on the entrance of the tide into a pool in which this sea-anemone is located. With this species expansion often begins within a few minutes after the arrival of the first new tidal water. As already pointed out I believe this expansion to depend upon the movement of the water and not upon its temperature or its oxygen content. Since the first water that enters the pool, often by indirect and not easily visible channels, may cause all the water in the pool to move somewhat, a stimulus imperceptible to the

observer may be given to every actinian there and thus induce expansion in what seems to be an anticipatory manner, whereas in reality it is a response to a direct stimulus. It is in some such way as this that in my opinion Piéron has probably been deceived, for from my own observations I am led to concur with Bohn ('08 a) in questioning the existence of reactions really anticipatory. Such rhythms as have thus far been studied in sea-anemones seem, therefore, to depend upon immediate rhythmic stimuli external in origin as the changes of the tides or the change from day to night and the reverse, and not upon rhythmic operations of a more internal nature such as probably control the pulsing of a jellyfish or the beat of the vertebrate heart.

4. PSYCHOLOGY

The term psychology as applied to such lowly animals as actinians is commonly used to cover a discussion of those activities which may or may not give grounds for the assumption of primitive psychic conditions in these forms. In this non-committal sense it is used here as it already has been used by many who have held most divergent views as to the problems involved. In all instances it implies a fundamental consideration of the more complex nervous processes of a given group of animals; a standpoint which it is quite appropriate to assume concerning the actinians. When we examine the organization of the human body and note the perfection of its voluntary adjustments with their involved psychoses and the equal perfection of such neuromuscular but non-psychic activities as those exhibited by the heart, we may reasonably ask, Is the actinian an organism that responds as the vertebrate heart does, or does it necessarily include in its activities elements of a psychic order? It is with questions such as these that the psychology of the actinians is concerned.

As Baglioni ('13) has pointed out, the neuromuscular reactions of actinians fall into two general classes, first, responses to beneficial stimuli, and, secondly, responses to noxious influences. These two categories doubtless represent the neural background on which rest those states of so-called pleasure and

pain that play so prominent a part in the central nervous activities of the higher animals (Holmes, '11). The best instance of the beneficial responses is that seen in feeding, and the noxious responses are well shown in general retraction and in locomotion, for in all actinians, so far as is known, locomotion is always away from the centre of stimulus. It is from a consideration of activities such as these that sound conclusions can be drawn concerning the possible presence of higher nervous operations in these animals.

From the standpoint of these general reactions the question of organic unity and centralization has already been discussed. The view clearly set forth by Jennings ('05) that the feeding actinian acts as a unit and that hunger and satiety are important elements in explaining the changes that appear in the course of its general responses seems to be quite unsupported by subsequent work. The essentially independent action of the tentacles as well as that of the reversing mechanism in the esophageal cilia and the discovery by Allabach ('05) that the changes in the whole mechanism as feeding proceeds are due to fatigue and not to anything comparable with satiety, a discovery with which my own observations are in accord, make clear that the feeding process is an activity which involves many semi-independent parts as such rather than the activity of the animal as a unit. When one contrasts the utter loss of effectiveness of the isolated appendages of higher animals with the almost normal activities of the detached tentacles of many actinians, the low degree of unity present in such forms as the sea-anemones becomes at once apparent. In the feeding of actinians each part reacts appropriately to its proper stimulus and the total act is carried out by a sequence of responses that have almost no relation to a central control. As already stated I agree thoroughly with Gee ('13) that the feeding reactions of actinians give no real support to the idea of organic unity in these animals.

General retraction is a response to conditions of an unfavorable or deleterious kind. It is an exhibition of the excessive tonicity of actinian muscle as pointed out by v. Uexküll ('09)

and by Jordan ('08). It involves the animal as a whole and yet it persists as strikingly in small fragments of an actinian as it does in the whole (Wolff, '04). Hence in this respect it gives no ground for the assumption of a specially unified state. The limp toneless condition of a muscle isolated from its nerve in a higher animal is in strong contrast to the tightly contracted fragment from an actinian's body.

The tidal and nychthemeral rhythms of these animals, as described by Bohn, are much more suggestive of organic unity than the single act of retraction itself. This is especially true when we take into account the retention of this rhythm after the removal of the rhythmic stimulus. Such activities imply the origin of new internal states through past stimulations and their retention in the subsequent modes of response of the animal as a whole. But it is by no means easy to judge of the value of Bohn's observations in these directions. His first description (Bohn, '06 c) of the rhythms and their retention was relatively simple, but his subsequent account shows such diversity and complexity that it is difficult for the reader to convince himself that rhythms have really been observed. Jennings ('09), who accepted Bohn's earlier observations with enthusiasm, was led in this way to entertain grave doubts about the accuracy of much that had been claimed. The fact that Piéron ('08 e) reexamined the question of the persistence of tidal rhythm in Actinia without being able to confirm Bohn's statement about it, and that Gee ('13) and I have been absolutely unable to find any evidence of retained rhythms, either tidal or nychthemeral, in the actinians that we have studied leads me to conclude that, though actinians may exhibit rhythms in consequence of rhythmic stimuli, they do not retain these rhythms on the disappearance of the stimuli. If retained rhythms do not occur in actinians, they can not of course be called upon as evidence of complex unified nervous states in these animals.

Intimately associated with the retention of rhythm in actinians is the retention of characteristic positional responses. Jennings ('05, p. 461) has shown that an Aiptasia will assume an irregular and distorted form in consequence of the irregularities

of the rocky cavity in which it has taken up its abode and that it will retain this shape for some time after it has been removed from its retreat. Moreover irregular forms of some permanence can be artificially and quickly produced by putting an animal into an artificial, irregularly shaped chamber. Such irregular forms are without much doubt due to differences in the degree to which tonicity is developed on particular parts of the animal's body by variously disposed stimuli. When it is remembered that the tonicity of general retraction may continue in some actinians even for days, it would not be surprising if an irregularly distributed tonicity should also have a lengthy period.

Van der Ghinst ('06) has also pointed out an interesting case of the retention of a characteristic positional response in Actinia. Specimens of this sea-anemone are found attached either to the undersides or to the uppersides of rocks. When individuals from both locations are collected and put in an aquarium in which both positions are possible, those that were originally on the underside of rocks reassume this position and those that were above move to the uppersides of objects. The positional relation apparently impressed upon them by their previous environment thus reasserts itself and in this manner gives evidence of modified central activities. The habit is said to be lost in twenty-four to forty eight hours.

The species of sea-anemones with which I have worked are not often found in the two positions assumed by Actinia and I have therefore not been able to carry out experiments on the lines worked on by Van der Ghinst. As no one seems to have repeated these observations on Actinia or other sea-anemones, and as Van der Ghinst himself claims for the retention of the response only the brief period of a day or so, it seems to me that they call for confirmation before they can be taken seriously into account in a discussion like the present one.

A third form of response which may be taken to involve the actinian as a whole is creeping. Locomotion by means of the pedal disc in these animals has already been rather fully discussed (Parker, '17 b) and it has been pointed out that this operation can be successfully carried out by specimens of Sa-

gartia from which the oral half has been cut away. Such fragments not only creep but creep away from the light as normal individuals do. In fact their activities are in no essential particular different from those of whole animals. Creeping, then, is in no sense dependent upon the animal as a unit but is an activity of the pedal disc and adjacent parts. It is, however, an activity of the disc as a whole. I have never been able to observe locomotion in pieces of the pedal disc. When actinians are cut in such a way that the fragments retain only parts of the original pedal disc, they remain attached to the substratum by their pedal surface but they never exhibit locomotion. It is only after regeneration has set in and a new pedal disc has been established that locomotion recommences. Creeping then is a response which calls for a much more unified mechanism than feeding and I agree with Lukas ('05, p. 126) in regarding it as a response which gives evidence of the highest form of nervous activity thus far discovered in actinians. I am, however, not prepared to go as far as he does and see in it evidence of a primitive form of desire and the earliest traces of consciousness (Lukas, '05, p. 127), but of its importance as indicative of a certain amount of unity in actinians there can be not the least doubt.

Another line of investigation that is suggestive of more than the simplest form of nervous activity in actinians is the modifiability of their responses. This subject has been justly emphasized by Jennings ('05), who has shown its significance by direct experiment. If a drop of water is allowed to fall on the surface of the water in which an expanded Aiptasia rests, the animal will usually retract. After expansion a second drop often fails to call forth any such response and in fact it is necessary to allow as a rule an interval of five minutes before a second response can be elicited. Thus the earlier stimulus influences the neuromuscular apparatus of the sea-anemone in such a way that a repetition of the stimulus is not followed by a response. To put the matter as Jennings does, the previous history of an organism has its influence upon its subsequent responses. This feature in actinians and in fact in most other animals has long been familiar to workers in this field, but it is to the credit of

Jennings to have insisted on its importance. When an explanation of this phenomenon is sought, one naturally turns, as in the case of the dying away of feeding responses, to exhaustion. Does not the initial stimulus, the vibration from the first drop of water, so exhaust the neuromuscular mechanism that it is incapable of receiving in an effective way a second stimulus till after a certain time for recovery?

This subject has been quantitatively studied by Kinoshita ('11), who has shown by the use of several kinds of weak stimuli that the response to the first stimulus is so considerable as compared with that to most subsequent stimuli that it is highly improbable that exhaustion plays any important part in the whole operation. Much more likely is it that the neuromuscular apparatus having responded once, assumes a state rather of adaptation than exhaustion and thus saves the organism from subsequent and useless responses. From this standpoint the condition left by the first stimulus and response seems to be that of inhibition—of the production of a refractory period so to speak—rather than that of exhaustion. At least it is clear that the first response has a relatively profound influence on the organism and that this influence lasts long enough—five or ten minutes—to affect subsequent stimuli. Here, then, in the truly nervous activities of actinians is evidence of the beginning at least of nervous states analogous to the more complex conditions found in higher forms.

In attempting to make clear the conditions under which the second or modified response takes place, care must be exercised that confusion does not arise as to the nature of the explanation. To one class of workers, those having a physico-chemical bent, a satisfactory explanation of the modified form of response would be found in an understanding of the interaction of the second stimulus and the receptor together with the chain of events that terminate in the muscular movements. This form of explanation is concerned exclusively with the working mechanism as such and has nothing to do with its historical origin. The second form of explanation, the one more likely to be adopted by those of a more biological turn, would seek for an understanding

of the modified response in the influences that had emanated from the original response and thus brought that response into historical relation with the second and modified one. This form of explanation emphasizes the effect of the history of the animal on its immediate state. Both forms of explanation have their places, but they are sufficiently diverse to require separation, a condition not always observed in discussions of this kind.

The opinion that the past history of an individual actinian is a potent factor in understanding its behavior has been expressed not only by Jennings ('05) but also by Piéron ('06 c, p. 15), who declared that the responses of actinians could not be looked upon as purely mechanical operations, but included traces of those activities characteristic of the central nervous organs of higher animals. But very little work has been done on actinians to ascertain the extent to which such central activities as those just indicated may extend. The limited range of response in these animals restricts such experimentation considerably. Heretofore associative processes have never been directly identified in actinians and my own efforts in this direction have always yielded negative results.

One of these attempts may be briefly described. When Metridium is slightly stimulated mechanically on the pedal edge of its column, it responds by a slight initial retraction. When food is put on the tentacles, the first response is an irregular but very characteristic waving of these organs in the immediate vicinity of the food. An attempt was made to associate the two stimuli mentioned so that the tentacle response might be called forth by a mechanical stimulation of the pedal edge of the column. A specimen of Metridium attached to a stone was placed in an aquarium of running seawater in a dimly lighted situation. After it was expanded it was mechanically stimulated and at the same time fed. These two stimuli were applied at the same time at intervals of half an hour from 9.30 in the morning till 4.30 in the afternoon. At each application the slight retraction and the initial waving of the tentacles were observed. After fifteen such trials the mechanical stimulus alone was applied but, though retraction was evident, no waving of the tentacles

accompanied it. On the following day a second series of trials was made on the same plan as the first except that the interval between stimulations was reduced from half an hour to a quarter of an hour. The trials began at 8.30 in the morning and extended to 1.30 in the afternoon. After 21 trials in which both mechanical and food stimuli were used together, the mechanical stimulus alone was applied. The animal retracted slightly as in all former applications but there was no waving movement of the tentacles. So far then as this form of experiment was concerned, there was no evidence of association.

From time to time other kinds of trials were made which, while they were not always directly concerned with association, were suggestive of higher nervous functions. None of these yielded positive results. One may be described. Sagartia luciae occurs commonly between tides and when exposed to the air it is retracted. If exposed for a long time, a day or more, it will dry up and eventually die from dessication. Specimens of this species that had been creeping actively on a sheet of glass were exposed to air, whereupon they retracted and dried slightly on their outer surfaces. When this resting state had been assumed, the glass was set in an inclined position in a vessel of seawater and at such a level that the lower pedal edge of one of these actinians was just in contact with the water, the rest of the animal being exposed to the air. Although it would have been easy for this particular actinian to have crept down into the water, it remained inactive and fixed to the spot for over three days while its mates on the part of the glass that was under water crept about freely. Similar trials on other individuals always gave the same results; the actinians remained fixed. Had they crept into the water, it would have required further experimentation to have ascertained the reason for this, but as they regularly did not, the observations give no grounds for the assumption of nervous activities of an exceptional order.

In examining the literature on the behavior of actinians two tendencies are quite obvious. One emphasizes the diffuse noncentralised nature of actinian responses and deals with the behavior of these animsls in terms of relatively simple reflexes

and the like; the other brings into prominence the unified action of these animals and interprets their behavior from the standpoint of the whole organism. It is by no means clear that these differences of opinion are really as pronounced as they sometimes appear on paper, but it is perfectly evident that the two general views represent real differences on a subject about which the truth can not at present be easily stated.

More or less of this difference is doubtless due to the various methods of attack which different investigators have used in this field of work. Since the external stimuli are more easily measured and otherwise controlled than the internal states, these were naturally first studied with the result that the work of Loeb, Nagel, and others led to a general conception of an actinian as a delicately adjusted mechanism whose activities were made up of a combination of simple responses to immediate stimulation. This view has been criticised by Jennings ('05, p. 448) as giving an unnaturally sharp, clear-cut and simple picture of actinian behavior. Jennings, moreover, has drawn attention to the physiological state of the animal including the effects of previous stimulation, of its metabolism and so forth, in fact of its general past history, as an internal element of no small importance in interpreting its reactions. Jennings' view has much to commend it, but, if assumed exclusively, it too has its limitations. As von Uexküll ('09, p. 74) and Baglioni ('13, p. 48) have recently pointed out, it too often tempts the worker to be satisfied with the statement of inferred internal states as explanations of conditions which upon careful scrutinizing prove to be dependent upon quite different factors, and the consequent vagueness and uncertainty with which it often surrounds the subject obscures the real questions for investigation. Keeping in mind these two tendencies, what can be said about the psychology of actinians? First of all it seems fairly certain that their behavior is chiefly determined by their immediate environment. They are expanded or retracted, feeding or quiescent, creeping or still in consequence of immediate stimulation rather than as a result of internal states due to past activities. They exhibit rhythmic responses only to immediate rhythmic stimuli,

not to the effects of past rhythmic stimulation. As a result of their various activities their physiological states are changed and these changes unquestionably modify their capacity for renewed response, but such changes are probably not very profound nor long lasting. From the evidence thus far accumulated, it appears that these states persist for only a brief period, often only a few minutes or at most some hours. It also appears that an actinian is much more nearly an organism whose internal state is one of general uniformity than one of great flux. On this uniformity as a background the changing environment calls forth now this now that set of responses without, however, seriously disturbing the internal equilibrium. This condition of affairs is in strong contrast with what is found in the higher animals, where the responses to the environmental influences are extremely diverse and variable in consequence of the internal states, and as a result these animals when subjected to experimental study often exhibit such novel and apparently unrelated responses that we are prone to speak of many of them as accidental or spontaneous. Spontaneity in this sense is not a characteristic of actinian behavior, which recalls very much more the relatively simple direct type of reaction as seen in such organs as the vertebrate heart. It is with this type of reactive mechanism rather than with the cerebral cortex that, in my opinion, the actinian shows affinity.

If such a view of the behavior of sea-anemones is correct, there is no reason to suppose that the unity of their organization is necessarily of a high order, as in fact the preceding accounts show. I am fully convinced that von Uexküll's description ('09, p. 75) of one of these animals as a bundle of reflexes is inadequate, but I am also fully convinced that this description is nearer the truth and certainly far freer from error than the picture drawn by Gosse ('60, p. 82) of these forms endowed with consciousness and will. The facts that the pedal half of an actinian may creep normally without the oral half and that the oral half is responsible for the feeding activities through a concourse of semi-independent parts, tentacles, esophagus, and so forth, make it quite obvious that the organic unity of the animal

as a whole is very weak. As Piéron ('06 a, p. 164) remarked, most parts of an actinian possess unusual autonomy. Or, to state the matter for the whole animal as von Uexküll ('09, p. 77) has phrased it for the nervous system, the sea-anemone partakes more of the nature of a sum of parts than of a unit. The harmony of action that is encountered on most sides in actinian behavior is in reality indicative of very little of that kind of unity that pervades the individual higher animal. To speak of sea-anemones as having a psychology is to use this term in its very broadest sense (Piéron, '06 a, p. 169).

5. SUMMARY

1. The appropriation of food in Metridium involves the mucous glands, the cilia, and the neuromuscular mechanism of the tentacles, as well as the esophageal cilia and the transverse mesenteric muscles.

2. Of these parts the neuromuscular apparatus of the tentacles is the only one the activity of which bears on the question of the action of the animal as a whole, and the changes which this mechanism shows are in all cases referable to sensory fatigue. Feeding therefore is not a unified activity, but is carried out by a concourse of semi-independent parts.

3. Retraction is produced by strong illumination and high temperature. Expansion by the presence of food and by water currents. Normal fluctuations in the amount of oxygen seems to have no direct effect on retraction and expansion.

4. Sagartia luciae exhibits in its retraction and expansion a well marked tidal rhythm; Metridium marginatum a well marked nychthemeral rhythm. Neither rhythm exhibited an anticipatory reaction and neither persisted after the removal of its rhythmic stimulus.

5. Actinians exhibit only a low degree of organic unity which is shown at its highest in their locomotion. They are organically more nearly a sum of parts than a unit.

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THE RHYTHMIC CONTRACTIONS IN THE MANTLE OF LAMELLIBRANCHS

ELIZABETH S. P. REDFIELD

FOUR FIGURES

INTRODUCTION

The respiratory currents through the mantle chamber of the lamellibranchs have been attributed exclusively to the action of the ciliated epithelium, covering the mantle, gills, and foot. Kellogg ('15) has recently made a thorough study of these currents and it appears from his work that they have a dual function; to supply water to the gills and to remove foreign matter from the mantle chamber.

Babak ('13, p. 197) has called attention to rhythmic movements of the shells of lamellibranchs, to which he attributes a cleansing function.

> Die Schalenbewegungen, welche zuweilen gleichsam rhythmisch und in auffälliger Frequenz erscheinen, haben (wenigstens bei Anodonta und Unio) kaum irgendwelche grössere respiratorische Bedeutung, da sie gerade im sauerstoffarmen Wasser nicht vermehrt werden, obwohl durch den auf diese Weise erfolgenden Wasserwechsel unzweifelhaft eine mächtige Förderung des Gaswechsels zustande kommen würde. Es lässt sich dartun, dass die Schalenbewegungen hauptsächlich als Reinigungs-reflexe aufzufassen sind.

This paper is a description of certain rhythmic movements found in the mantle of several species of lamellibranchs, and of experiments to determine the function of these movements. The recent summary of work on the respiration of lamellibranchs by Babak ('12) makes no mention of this phenomenon, which apparently thus far has passed undetected.

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DESCRIPTION OF THE MOVEMENTS OF THE MANTLE

If a small hole is cut in the shell of a clam (Unio complanatus), the mantle will bulge out through it. At regular intervals the mantle may be observed to contract rapidly and then to bulge out slowly. To determine the periods of time between these contractions, a small bent needle was hooked through the mantle and attached by a silk thread to an aluminum lever and records were taken on a kymograph. Several species of lamellibranchs were tested and the rate of the rhythm was found to vary in them. It also varied in different individuals of the same species. In some the period was about one minute, in others about three. In Mya arenaria it was most uniform. The periods lengthen after a clam has been kept in the laboratory for several weeks. It was necessary therefore to use only clams which had been collected within a short time before the tests were made.

The movement of the mantle might be attributed to changes of pressure within the mantle chamber, produced by the rhythmic movements of the shell, such as Babak has described. In order to determine this point the following experiment was carried out. Simultaneous records were taken of the movements of both the shell and the mantle of a Unio complanatus. Figure 1, *A*, *B*, shows these movements to be in unison. The parts of the valve of this clam to which the anterior and posterior adductor muscles were attached were now cut free from the rest of the valve, thus allowing the adductor muscles to contract without moving the rest of the valve. Records were again taken simultaneously of the movements of both the shell and mantle (fig. 1, *C*, *D*). It is clear from these records that, although the shell was motionless, a slight but distinct contraction of the mantle occurred at regular intervals.

During the summer of 1915 the movements of the mantle in a number of marine lamellibranchs were studied at the laboratory of the United States Bureau of Fisheries at Woods Hole, Mass.

In Mya arenaria the shell may be held tightly closed without disturbing the flow of water through the mantle chamber, for the siphons can be extended when the valves are in this position. The pulsations of the mantle in Mya arenaria are much more powerful than those in Unio complanatus. A wave of contraction can be seen to start in the distal end of the extended siphon and move forward ending with the rise and fall of the mantle. This recalls an observation of Dubois ('92) on Pholas

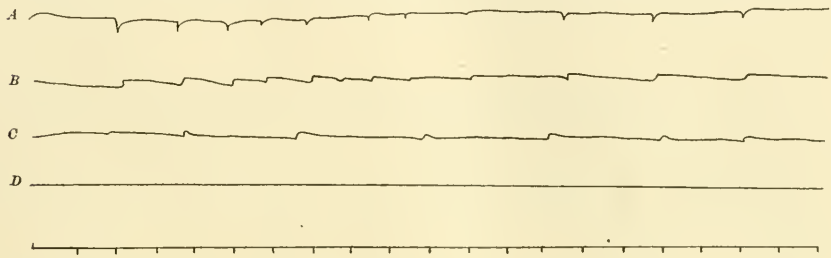

Fig. 1 Kymographic records of the movements of the mantle and shell of a Unio complanatus. The upper line (*A*) is a record of the movements of the mantle; the second line (*B*) is a record of the movements of the shell. Both records were made simultaneously.

The third line (*C*) is a record of the movements of the mantle; the fourth line (*D*) is a simultaneous record from the shell of the same specimen after the shell had been rendered immobile by cutting away from the rest of the valve the part of the shell which was attached to the adductor muscles.

The lower line indicates time intervals of thirty seconds each.

dactylus; a wave of muscular action in the walls of the siphons served as a means of circulating water when the valves of the animal were tightly closed.

Figure 2, *A*, is a record of the mantle movements of Mya. It shows that the interval between successive contractions is about one minute, with often a longer pause after three contractions. In table 1, is shown the rate of mantle and shell contractions of Mya arenaria kept in running water during a period of three days. This table indicates that the rate varies considerably from time to time.

Figure 3 is a record of the movements of the mantle lobes of both sides of a Mya arenaria taken simultaneously. It will be

observed that the contractions of the two sides are in most cases synchronous. By contracting synchronously the lobes would tend to decrease the size of the infra-branchial chamber and consequently drive some of the water out of it.

In ten other species of marine lamellibranchs pulsations of the mantle of varying degrees of intensity were noted. In those

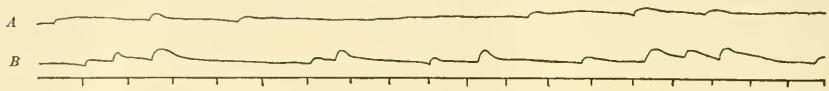

Fig. 2 Kymographic record of the movements of the mantle of Mya arenaria, the valves of which have been held tightly closed. The upper line (*A*) is a record taken while the clam was in running water. The second line (*B*) is a record taken thirty minutes after the water in which the clam was placed had been covered with paraffin oil. It illustrates the accelerated rate which characterizes the early stages of suffocation. The lower line indicates time intervals of thirty seconds each.

TABLE 1

The rate of contraction of the mantle and shell of a Mya arenaria kept in running water for three days. Averages are based on counts extending over twenty-minute periods

DATE	TIME	AVERAGE NUMBER OF CONTRACTIONS OF MANTLE PER MINUTE	AVERAGE NUMBER OF CONTRACTIONS OF SHELL PER MINUTE
First day	9.30 a.m.	1.3	0.2
First day	10.00 a.m.	1.3	0.35
First day	11.00 a.m.	1.3	0.1
Second day	5.30 p.m.	0.9	0.15
Third day	6.30 p.m.	0.5	0.3
Third day	7.15 p.m.	0.9	0.25

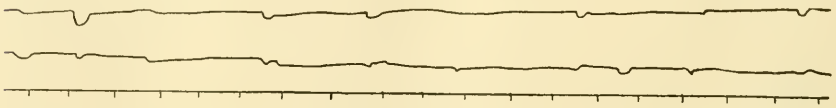

Fig. 3 Simultaneous kymographic records of the movements of the right and left mantle lobes of Mya arenaria with the shell tied closed. The top line is a record of the left mantle lobe; the middle line is a record of the right mantle lobe. The bottom line indicates time intervals of thirty seconds each.

These records illustrate the fact that the two lobes contract synchronously.

forms which are sedentary, the rhythmic movements were found to be much as in Mya arenaria. Such forms were Modiolus modiolus, Modiolus plicatula, and Mytilus edulis. The last showed only weak movements. In the forms which were active, such as Solemya velum, Ensis directis, Cummingia tellinoides, and Yoldia sapotilla, there are also movements of the mantle perhaps respiratory in function, but somewhat different from those in Mya. In Pecten gibbus, Venus mercenaria, and Ostrea virginica the mantle is very thin and in close connection with the shell. No movements of the mantle were discovered in these forms. The gills in the last three are more active than those in the others, which may be of significance.

THE FUNCTION OF THE MOVEMENTS OF THE MANTLE

To determine whether the movements of the mantle were concerned with setting up the respiratory currents in the infrabranchial chamber, the effect of suffocation upon the rate of the rhythm was determined. Specimens of Mya, placed in a bowl of running sea-water, were attached to the recording apparatus and the rate of the mantle pulsations determined. The water was then covered with a thin layer of paraffin oil.[1] As the oxygen in the water became exhausted the rate of the rhythm increased rapidly (fig. 2), but as the movements proceeded this rate fell off, the movements stopping completely before the clam succumbed. Figure 4 is a graphic representation of the changes in the rate of movements of the mantle of Mya arenaria during suffocation.

If, as the foregoing facts suggest, the mantle movements are concerned with setting up respiratory currents, it might be expected that an obstruction of these movements would influence the quantity of oxygen consumed by the clam. Accordingly experiments were carried out in which the oxygen consump-

[1] Bayliss ('15) has pointed out the futility of attempting to preserve solutions from the action of gases in the atmosphere by covering them with oil or hydrocarbons. This observation does not invalidate the results of the experiments in question since the animals died of suffocation, though perhaps no more rapidly than if the surface of the water had not been covered with paraffin oil.

tion by fresh clams was compared with the oxygen consumption of the same animals in which the movement of the mantle had been checked.

Several liters of water drawn from the tap were left standing in a closed vessel for twenty-four hours in order that the oxygen content might come to a uniform condition. The amount of oxygen in a sample of this water was then determined by the Winkler method as used by Birge and Juday ('11). A 350 cc. glass jar containing a well cleaned Unio was filled with this

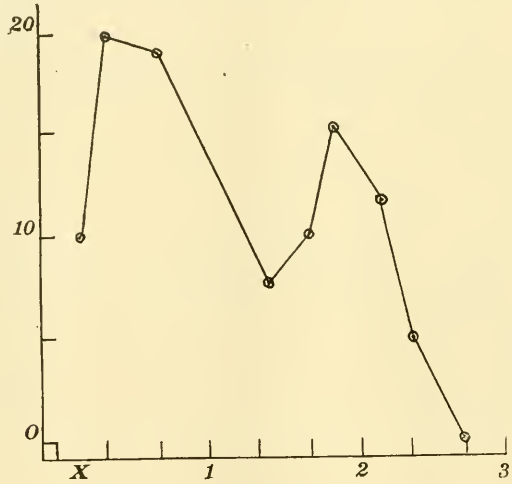

Fig. 4 The solid line indicates the rate of contractions of the mantle of Mya arenaria during suffocation. Time is indicated in hours along the abscissa; the number of movements of the mantle in twenty minutes is measured along the ordinate. Suffocation was commenced at *X*.

water and closed with a tight-fitting ground-glass cover. The jar was allowed to stand twenty-four hours and then a 250 cc. sample was siphoned off and the amount of its contained oxygen was determined. This value subtracted from the original oxygen content of the same volume of water gave the amount of oxygen consumed by the clam in twenty-four hours from that volume.

Next, the mantle of the same clam was made functionless by inserting a small knife between the ventral edges of the valves and slitting the mantle on both sides longitudinally from one

end to the other. To overcome whatever shock might follow and disturb the normal behavior of the organism, the clam was put in fresh water and left for six to eight hours. This clam was then placed in a freshly filled jar and the oxygen consumed by it in twenty-four hours again determined. Table 2 gives the results of a series of such experiments. From this table it is clear that the oxygen consumption of the clams in which the action of the mantle has been checked is greatly reduced. This fact seems to me to show that the mantle in Unio complanatus is concerned with the respiratory function.

TABLE 2

Comparison of the quantity of oxygen consumed by specimens of Unio complanatus before and after the activity of the mantle had been checked

UNIO COMPLANATUS	CUBIC CENTIMETERS PER LITER OF OXYGEN USED IN 24 HRS. BY CLAM WITH MANTLE INTACT	CUBIC CENTIMETERS PER LITER OF OXYGEN USED IN 24 HRS. BY *same* CLAM WITH THE MANTLE ACTIVITY DESTROYED
1	5.671	4.016
2	5.495	1.516
3	5.584	1.906
4	4.918	1.994
5	5.986	2.316
6	3.950	2.610

It may be objected, however, that the injury caused by the operation may have lowered the vitality of the clam, or caused such a loss of blood as to have produced death. To determine whether this objection had any weight or not, clams were subjected to numerous other operations. The distal ends of the siphons were cut off, the foot was mutilated, the mantle cut only on one side, or numerous small holes were made in it. Clams thus operated on lived: some a week, others several weeks, others even months. As table 3 shows, these operations had almost no retarding effect on respiration.

If fresh clams (Unio complanatus) are left in a sealed jar containing 300 cc. of water, death occurs in not less than four days. If the movements of the mantle in clams be checked as described in the foregoing experiment, death occurs in twenty-

TABLE 3

Comparison of the quantity of oxygen consumed by specimens of Unio complanatus before and after being mutilated in various ways

UNIO COMPLANATUS	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY A CLAM WITH SIPHONS INTACT	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY SAME CLAM WITH SIPHONS CUT OFF
1	3.841	4.206
2	4.985	3.148
	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY A CLAM WITH THE MANTLES INTACT	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY SAME CLAM WITH A SMALL HOLE CUT IN MANTLE OF ONE SIDE
1	5.688	4.256
2	6.043	3.130
	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY A CLAM WITH THE MANTLES INTACT	CUBIC CENTIMETERS PER LITER OF OXYGEN USED BY SAME CLAM WITH MANTLE OF ONE SIDE DESTROYED
1	4.529	4.158
2	4.128	2.086

four hours. This fact indicates that the clams on which operations have been performed are unable to avail themselves of the oxygen which is in the water surrounding them.

SUMMARY

1. A rhythmic movement of the mantle, independent of movements of the shell, is described in a number of marine and freshwater lamellibranchs.

2. Experiments are detailed which indicate that the rate of these movements increases during the early stages of suffocation, that the oxygen consumption of the clams is decreased by checking completely these movements, and that clams suffocate more rapidly under these circumstances than otherwise.

3. It is concluded that the movements of the mantle in lamellibranchs is an important factor in setting up the respiratory currents. The movements may also be of significance in driving waste materials and foreign bodies from the mantle chamber.

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THE EXPERIMENTAL MODIFICATION OF GERM CELLS. III. THE EFFECT OF PARENTAL ALCOHOLISM, AND CERTAIN OTHER DRUG INTOXICATIONS, UPON THE PROGENY[1]

RAYMOND PEARL

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SEVEN FIGURES

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1. INTRODUCTION

In this paper it is proposed to discuss the effects, so far as any are observable, of the alcoholization of one or both of the parents upon the progeny in the first generation using the domestic fowl as material. Different characters of the progeny will be considered, and as before primary attention will be given to such characters as admit of quantitative expression.

A detailed account of the experimental methods used, the specific problems attacked, the breeding of the foundation stock

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station. No. 102.

used, etc., has been given in I,[2] the first paper in this series. The effect of the alcoholization by the inhalation method used, upon the treated birds themselves, has been given in II.[3]

It will be recalled that, as stated in I, the present report covers the results of the work up to February 1, 1916 only. Further data will be given in later reports.

II. THE FERTILITY AND HATCHING QUALITY OF THE EGGS FROM ALCOHOLIZED PARENTS

One of the surest and most delicate indicators of constitutional vigor and vitality in poultry which has yet been discovered is the hatching quality of the eggs. Anything which upsets the general metabolic balance or impairs the vitality of either partner in a mating will show its effect in a diminished hatching power of the eggs from that mating. In view of these facts an examination of the data relative to this character in these alcoholic matings becomes of especial interest.

Before entering upon such an examination it is necessary to consider the question of control data. Unfortunately the untreated control male No. 666 (cf. I, table 2) proved to be practically completely impotent sexually. He mated regularly and apparently effectively with the females in his pen, but practically all of the eggs proved to be infertile, regardless of whether the female concerned was an alcoholic or an untreated normal control. In consequence of this matings 2131, 2132, and 2133 which were planned to serve as controls, were practically complete failures. The results which they gave were so far from normal that it would be entirely misleading to use them as con-

[2] This refers to the first paper in this series, which was entitled: "The experimental modification of germ cells. I. General plan of experiments with ethyl alcohol and certain related substances." Jour. Exp. Zool., vol. 22, pp. 125. Throughout this and the later papers in the series cross-references to other papers in the same series will be made simply by the Roman numeral designating the paper referred to, together with the particular page number to which reference is made.

[3] This refers to the second paper in this series, which was entitled: "II. The effect upon the domestic fowl of the daily inhalation of ethyl alcohol and certain related substances." Jour. Exp. Zoöl., vol. 22, pp. 165.

trols on the alcoholic matings. When ♂666 was autopsied the testes were found to be very small, much under size for a bird of his age and body weight. He was apparently a naturally impotent male, of a sort that occurs not infrequently in poultry breeding operations. Owing to his deceptively vigorous behavior it was too late in the year to substitute another bird in his place when it was finally proven that he was worthless as a breeder.

In consequence of the failure of this ♂666 we are compelled to resort to other data to furnish proper controls for the fertility and hatching data. Fortunately such data are at hand from other experiments, and are entirely adequate both in kind and amount. For comparison with the results of the alcoholic matings there will be used in this section of the paper the results from a random sample, comprising 22 matings, from all of the 1915 matings of normal untreated birds in which the female partner was a pure Barred Plymouth Rock. A sample of 22 matings is taken because that is the number of matings in which one or both of the animals involved was a treated bird.

The data for the treated matings are given in table 1. The arrangement and meaning of the captions of this and the next table need some explanation. In the column headed 'Eggs set' is given for each mating the total number of eggs which went into the incubator from that mating. The percent of infertile eggs (5th column) is calculated on this total. An infertile egg is one in which there has been no union of sperm and ovum, i.e., no zygote formation. In the 'Died in shell' column is given the number of embryos which, having started development, died before actually hatching. The 'Per cent of embryos dying in shell' is calculated on the basis of the number of fertile eggs, according to the following formula:

$$\frac{100\ (\text{Died in shell})}{(\text{Eggs set}) - (\text{Infertile})} = \text{Per cent of embryos dying in shell}$$

Other captions are self explanatory.

The control data for comparison are given in table 2.

From these tables the following points are to be noted:

1. All of the treated males were clearly entirely potent. This is proved by the fact that certain of the matings in each sub-

TABLE 1

Data on the fertility and hatching of eggs from treated parents in 1915

NATURE OF MATING	MATING NUMBER	EGGS SET	INFERTILE	PER CENT INFERTILE	DIED IN SHELL	PER CENT OF EMBRYOS DYING IN SHELL	HATCHED	PER CENT FERTILE EGGS HATCHED	PER CENT ALL EGGS HATCHED
Ethyl ♂ × untreated ♀	2116	33	17	51.5	7	43.8	9	56.3	27.3
	2117	20	2	10.0	4	22.2	14	77.8	70.0
	2118	25	0	0.0	4	16.0	21	84.0	84.0
	2119	33	4	12.1	22	75.9	7	24.1	21.2
Totals: Ethyl sire only		111	23	20.9	37	42.0	51	58.0	45.9
Ethyl ♂ × ethyl ♀	2112	28	15	53.6	2	15.4	11	84.6	39.3
	2113	24	2	8.3	4	18.2	18	81.8	75.0
	2114	25	20	80.0	5	100.0	0	0.0	0.0
	2115	27	19	70.4	2	25.0	6	75.0	22.2
Totals: Ethyl sire and dam		104	56	53.9	13	27.1	35	72.9	33.7
Grand totals: Ethyl ♂		215	79	36.7	50	36.8	86	63.2	40.0
Methyl ♂ × untreated ♀	2123	28[1]	18	64.3	5	50.0	5	50.0	17.9
	2124	32	14	63.6	3	37.5	5	62.5	22.7
	2125	38[1]	9	23.7	2	7.4	26	92.9	68.4
Totals: Methyl ♂ only		88	41	46.6	10	21.7	36	78.3	40.9
Methyl ♂ × methyl ♀	2120	34	32	94.1	1	50.0	1	50.0	2.9
	2121	24	9	37.5	3	20.0	12	80.0	50.0
	2122	27	26	96.3	0	0.0	1	100.0	3.7
Totals: Methyl sire and dam		85	67	78.8	4	22.2	14	77.8	16.5
Grand totals: Methyl ♂		173	108	62.4	14	21.9	50	78.1	28.9
Ether ♂ × untreated ♀	2104	32	0	0.0	15	46.9	17	53.1	53.1
	2109	24	11	45.8	7	53.1	6	46.2	25.0
	2110	30	4	13.3	15	57.7	11	42.3	36.7
	2111	29	0	0.0	2	6.9	27	93.1	93.1
Totals: Ether ♂ only		115	15	13.0	39	39.0	61	61.0	53.0
Ether ♂ × ether ♀	2105	35	34	97.1	1	100.0	0	0.0	0.0
	2106	25	4	16.0	3	14.3	18	85.7	72.0
	2107	22	6	27.3	8	50.0	8	50.0	36.4
	2108	22[1]	7	31.8	3	21.4	11	78.6	52.4
Totals: Ether sire and dam		104	51	49.0	15	28.8	37	71.2	35.6
Grand totals: Ether ♂		219	66	30.1	54	35.3	98	64.1	44.7
Grand totals: all treated ♂♂ × untreated ♀♀		314	79	25.2	86	36.6	148	63.0	47.1
Grand totals: all treated ♂♂ × treated ♀♀		293	174	59.2	32	26.9	86	72.3	29.4

[1] One egg broken during incubation.

TABLE 2

Data on the fertility and hatching of eggs from random sample of untreated BPR ♀♀ × untreated ♂♂ in 1915

MATING NUMBER	EGGS SET	INFERTILE	PER CENT INFERTILE	DIED IN SHELL	PER CENT OF EMBRYOS DYING IN SHELL	HATCHED	PER CENT FERTILE EGGS HATCHED	PER CENT ALL EGGS HATCHED
1956	27	16	59.3	6	54.5	5	45.5	18.5
1957	28	15	53.6	8	61.5	5	38.5	17.9
1958	39	0	0.0	15	38.5	24	61.5	61.5
1959	17[1]	8	47.1	8	100.0	0	0.0	0.0
1960	27	1	3.7	5	19.2	21	80.8	77.8
1961	38	2	5.3	3	8.3	33	91.7	86.8
1962	41	20	48.8	15	71.4	6	28.6	14.6
1964	36	2	5.6	12	35.3	22	64.7	61.1
1965	31	6	19.4	5	20.0	20	80.0	64.5
1968	35[1]	0	0.0	15	44.1	19	55.9	55.9
1971	31	5	16.1	12	46.2	14	53.8	45.2
1972	33	0	0.0	18	46.7	15	53.3	53.3
1973	40	11	27.5	4	13.8	25	86.2	62.5
1974	37	37	100.0	0	0.0	0	0.0	0.0
1983	20	7	35.0	5	38.5	8	61.5	40.0
1984	22	1	4.5	3	14.3	18	85.7	81.8
1985	36	12	33.3	18	75.0	6	25.0	16.7
1986	36	3	8.3	24	72.7	9	27.3	25.0
1987	29	0	0.0	12	41.4	17	58.6	58.6
1988	45[1]	3	6.7	14	31.8	27	68.2	61.4
1989	28	11	39.3	7	41.2	10	58.8	35.7
1991	41	6	14.6	22	62.9	13	37.1	31.7
Totals..	717	166	23.3	231	42.2	317	57.8	44.4

[1] One egg was broken during incubation.

division of table 1 show a high degree of fertility. Thus in the case of the ethyl ♂ mating 2118 gave no infertile eggs out of a total of 25 eggs set. Out of these 25 eggs were hatched 21 good chickens. In the case of mating 2113, with an ethyl ♀, this same ethyl ♂ had but 2 infertile eggs out of a total of 24 set. Anyone who has had experience with poultry knows that records of this sort are not to be obtained with impotent or defective males. The methyl ♂ made no record quite so good as these of the ethyl ♂ in respect to fertility of eggs. Mating 2125, however, with 9 eggs infertile out of 37 set shows that the male was not seriously defective in his ability to fertilize eggs.

The ether ♂ has two records of perfect fertility with untreated females (matings 2104 and 2111). With an ether ♀ he gave, in mating 2106, only 4 eggs infertile out of a total of 25 set.

2. Considering totals and averages it is clearly evident that the inhalation treatment of the females seriously reduces the proportion of fertile eggs which they are capable of producing. Taking grand totals 59.2 per cent of all eggs set were infertile when the mating was of the type "treated ♂ × treated ♀," as against 25.2 per cent infertile for matings of the type "treated ♂ × normal, untreated ♀," and 23.2 per cent for matings of the type "normal, untreated ♂ × normal, untreated ♀." It thus appears that the treated females gave rather more than twice as many in fertile eggs as untreated females in the same season and under the same conditions except for the alcoholic treatment. Calculating the correlation coefficient between germ dosage index and percentage of eggs infertile we get

$$r = +0.316 \pm 0.136$$

This is a relatively large coefficient and almost certainly significant. It means that the higher the germ dosage the greater the proportionate failure of the germ cells to form zygotes. This defect in the percentage of fertility in treated females' eggs might, on *a priori* grounds, conceivably be due to any one of three general sets of causes, viz., (a) that the oviduct of the treated females formed a less favorable environment for the sperm than the oviduct of the normal female, or (b) that the eggs of the treated females were themselves adversely affected by the alcohol, so that in respect either of their chemical or physical condition or both they were less capable of being fertilized than the eggs of normal untreated females, and at the same time the sperm of treated males was less capable of fertilizing, or (c) that there was an assortative mating operating against treated females, and in favor of normal, untreated females in the same breeding pen. There appears to be no doubt that the principal cause of the reduced fertility is the second one mentioned (b). The correlation between germ dosage and infertility indicates that a certain proportion of the germ cells which

would form zygotes under normal circumstances are definitely put out of commission by the treatment. While such inactivation of germ cells is undoubtedly the primary factor in reducing the fertility, the other two factors also play some part.

All the evidence at hand from observations on the behavior of these birds indicates that factor (c), preferential mating in favor of untreated females, is second in importance to (b) in reducing the percentage of fertility of the eggs of treated females. Observations of the males in the breeding pens indicate that the alcoholized females are not sought by the males with either the eagerness or the frequency that the untreated females are. Just what is the basis of this preference does not yet appear. Factor (a) probably plays a minor part in the diminished fertility of the eggs of the treated females.

4. Comparing the different males it appears that, taking the data from all matings both with treated and untreated females, the ether ♂ gave the highest fertility (69.9 per cent fertile), the ethyl ♂ the next highest (63.3 per cent fertile), and the methyl ♂ considerably lower (37.6 per cent fertile). All of these figures are lower than the mean of the random sample of 22 normal matings of untreated males with untreated B. P. R. females recorded in table 2. Those matings gave 76.8 per cent fertile eggs. There appears to be no doubt that the process of zygote formation (fertilization) in these treated matings was significantly impaired by the treatment in every case. This impairment was most serious in matings of the methyl ♂. His best mating (2125) gave a percentage of fertility, 76.3 per cent slightly lower than the average for a random sample of matings of untreated matings in the same season. All cross-bred matings of untreated males and females in 1915 gave 536 eggs infertile out of 2628 set, or 79.6 per cent fertile. The methyl ♂'s percentage is significantly below this.

5. The percentage of fertile eggs hatched is higher for the matings of treated males, whether mated with treated or untreated females, than for the random sample of normal matings in which both parents are untreated. It is also higher in these treated matings than in all cross-bred matings in 1915, which

gave 1122 chickens from 2092 fertile eggs, or 53.6 per cent. The figures for the treated matings, 63.0 per cent for treated ♂♂ × untreated ♀♀, and 72.3 per cent for treated ♂♂ × treated ♀♀, are significantly higher than the normal hatching records of the same year. These figures appear to demonstrate that the alcohol (or ether) treatment had no deleterious effect upon the hatching quality of the eggs of treated individuals. On the contrary the fertile eggs of such individuals hatched distinctly better than eggs of normal individuals. The correlation between total germ dosage index and per cent of fertile eggs hatched gave

$$r = +0.288 \pm 0.138$$

On account of the small series the probable error is large and too much stress can not be laid upon the result. On the other hand, small as the series is, the coefficient is more than twice its probable error, and is to be regarded as probably significant. So taken, it means that the larger the germ dosage the larger the proportion of embryos which hatched, or, put the other way about, the smaller the prenatal mortality.

6. The last column of each table gives the percentage of chicks hatched to all eggs set. For matings of treated ♂♂ × untreated ♀♀ the final average percentage is 47.1. This is a higher proportion of chicks hatched to eggs set than is given either by the random sample of normal matings in table 2 (44.4 per cent), or by all cross-bred matings of 1915, in which 1122 chicks, or 42.7 per cent, were hatched from 2628 eggs set. These figures show that in spite of the lower zygote forming power in the treated matings, which has already been pointed out, the hatching power of the eggs was enough higher than the normal to bring the net hatching percentage above that for normal, untreated birds.

7. For matings of treated ♂♂ × treated ♀♀ the case is different. Here but 29.4 per cent of all eggs set produced chickens. The excess hatching power in the eggs of this group was not sufficient to offset the very poor fertility.

8. The highest average percentage of chicks hatched to all eggs set was from the matings of ether ♂ × untreated ♀♀ the lowest from the matings of methyl ♂ × methyl ♀♀.

Summarizing the general features of the above results regarding production of offspring by alcoholized parents it may be said that the average fertility of eggs (i.e., proportion of zygotes formed) is diminished and the average hatching power of the fertile eggs is increased after alcoholization of the parents. The reduction in average fertility of the eggs is due primarily to the effect on the germ cells, and in some part to the fact that alcoholized females are not as attractive to the males as untreated, and hence are discriminated against in the matings, and furthermore probably in some part to the fact that the oviduct of the treated female does not furnish so favorable an environment for sperm as the oviduct of untreated females. The net result is that alcoholized parents produce on the average fewer offspring per mating unit than do normal, untreated parents under conditions otherwise similar.

In all these results on the production of offspring there is no definite evidence that any deleterious blastophthoric effect has been produced by the poisoning upon the germ cells which produce zygotes. On the contrary what we do have is clear evidence that the treatment acts as a selective agent on the germ cells, entirely eliminating from zygote formation the weaker and less vigorous. The gametes which survive this selective process and take part in zygote formation are the most vigorous and resistant part of the germ cell population. Their superiority is clearly demonstrated in the significantly higher per cent of fertile eggs hatched when both parents are treated. This means that the embryos formed by these germ cells are superior in vigor and vitality to embryos formed by an unselected sample of germ cells as in normal reproduction. As we proceed we shall see that this initial superiority is maintained throughout the life history of the offspring of treated parents. The quantitative analysis of the different factors concerned in the reproductive process, which has been presented in this section, taking into account the correlations, constitutes convincing evidence that the poisons used act as selective agents on the germ cell population.

Other workers have noted adverse effects of poisons upon production of offspring. Stockard and Papanicolaou (38[4], p. 172) report with guinea pigs about 15 per cent more successful matings with normal than with alcoholic parents. They also found a higher proportion of still-born young in the matings of alcoholic parents than in controls. Weller (42 p. 292) finds that chronic lead poisoning in guinea pigs leads in some cases to sterility of the male without loss of sexual activity, and in case the female is poisoned, to an increase in the number of still-births. Cole and Bachhuber (3, p. 27) present some evidence regarding the effect of lead poisoning of the parents upon the fertility and hatching quality of eggs. In their experiments the percentage of fertile eggs from the control, untreated male is abnormally low, so that it is impossible to say whether the percentage given by the treated male (73 per cent fertile) is to be regarded as above or below the normal average fertility of untreated males for that particular season and strain of birds. Probably it was below, thus agreeing with the data here presented. On the other hand, the percentage of fertile eggs hatched was nearly twice as high in the case of the normal untreated sire as in the case of the sire poisoned with lead acetate. Lead appears to have a different action on fowls, in this respect, than do the substances used in the present experiments, alcohol and ether.

III. TOTAL BREEDING CAPACITY

In the preceding section the quantitative results concerning the production of offspring by alcoholized parents have been treated in detail. From these detailed figures it is somewhat difficult to form a general comprehensive idea of the reproductive capacity of these birds taking all the pertinent factors into account together. One question for which we want an answer is this: What relation, if any, is there between the total germ dosage index for each mating and the total reproductive capacity of that mating?

[4] Throughout this paper the references to literature are by number and refer to the bibliography printed at the end of I. By this method space is saved over what would be involved in reprinting the entire bibliography here.

An adequate method of collating and digesting the data in such a way as to make an answer to these questions possible appears to be afforded by the 'poultry selection index' devised by Pearl and Surface (27, p. 392) some years ago. This index is described in this way:

> The formula which has been provisionally adopted in our work as a fundamental poultry selection index is as follows:
>
> $$I_1 = \frac{5\,(a + b)}{c + d + 1}$$
>
> The following scheme shows the meaning of the letters in the formula:
> I_1 = general or fundamental poultry selection index for an individual bird.
> a = percentage of this bird's fertile eggs which hatched.
> b = percentage of eggs actually laid by this bird to the total number it was possible for her to lay between February 1 and June 1 (i.e., the breeding season) of the year for which the index is calculated.
> c = percentage of this bird's eggs which were infertile.
> d = percentage of chicks which died within three weeks from the date of hatching.

In the present connection, and probably generally, it is better to designate this constant as the 'poultry breeding index' rather than 'selection' index. The values of the poultry breeding index for the 1915 alcoholic matings are set forth in table 3, together with the subsidiary values on which they are based. In the case of matings 2105 and 2108 inclusive, in which the ether females were involved, it will be recalled from table 2 that these birds were killed on April 15 by too long a stay in the tank. In calculating the breeding index for these birds the factor b in the formula for index has been taken as the percentage of eggs actually laid by each bird to the total number it was possible for her to lay between February 1 and April 15.

From this table it is evident that there is a good deal of variation in the breeding index in the different matings. The constant ranges in value from 1.3 to 99.6. The highest value tabled by Pearl and Surface for this index was 45.2.

The relation between total reproductive capacity as indicated by the index, and the total germ dosage with alcohol is shown graphically in figure 1. The total germ dosage index

TABLE 3

Breeding index for treated matings in 1915

NATURE OF MATING	MATING NUMBER	*a*	*b*	*c*	*d*	*I*
Ethyl ♂ × untreated ♀	2116	56.3	64.2	51.5	33.3	7.0
	2117	77.8	40.0	10.0	7.1	32.5
	2118	84.0	66.7	0.0	28.6	25.5
	2119	24.1	70.8	12.1	71.4	5.6
Ethyl ♂ × ethyl ♀	2112	84.6	61.7	53.6	18.2	10.0
	2113	81.8	66.7	8.3	0.0	79.8
	2114	0.0	46.7	80.0	100.0	1.3
	2115	75.0	55.8	70.4	50.0	5.4
Methyl ♂ × untreated ♀	2123	50.0	66.7	64.3	0.0	8.9
	2124	62.5	49.2	63.6	0.0	8.6
	2125	92.9	76.7	23.7	7.7	26.2
Methyl ♂ × methyl ♀	2120	50.0	61.7	94.1	0.0	5.9
	2121	80.0	65.8	37.5	0.0	18.9
	2122	100.0	44.2	96.3	0.0	7.4
Ether ♂ × untreated ♀	2104	53.1	65.8	0.0	29.4	19.6
	2109	46.2	56.7	45.8	50.0	5.3
	2110	42.3	62.5	13.3	18.2	16.1
	2111	93.1	74.2	0.0	7.4	99.6
Ether ♂ × ether ♀	2105	0.0	60.3	97.1	100.0	1.5
	2106	85.7	72.6	16.0	5.6	35.0
	2107	50.0	41.1	27.3	0.0	16.1
	2108	78.6	28.8	31.8	30.0	8.6

as given in table 6 of I, is plotted as a solid line starting with its lowest point at the left hand side of the diagram and ascending continuously, but at changing rates. The breeding indices for the same matings, as given in table 3, are plotted as a dash line. Now if it were a fact that the total reproductive capacity (as here defined) of the bird in these matings diminished with increasing total dosage of the gametes with alcohol or ether, it clearly would be the case that the dash line would tend to have its highest points at the left hand end of the diagram and its lowest points at the right hand end. Or, in other words, as the germ dosage line went up the reproductive capacity index line would tend to go down. As a matter of fact it is obvious that nothing of the sort happens. The breeding index line is essentially horizontal, neither going up nor down as the value of the total germ dosage index rises.

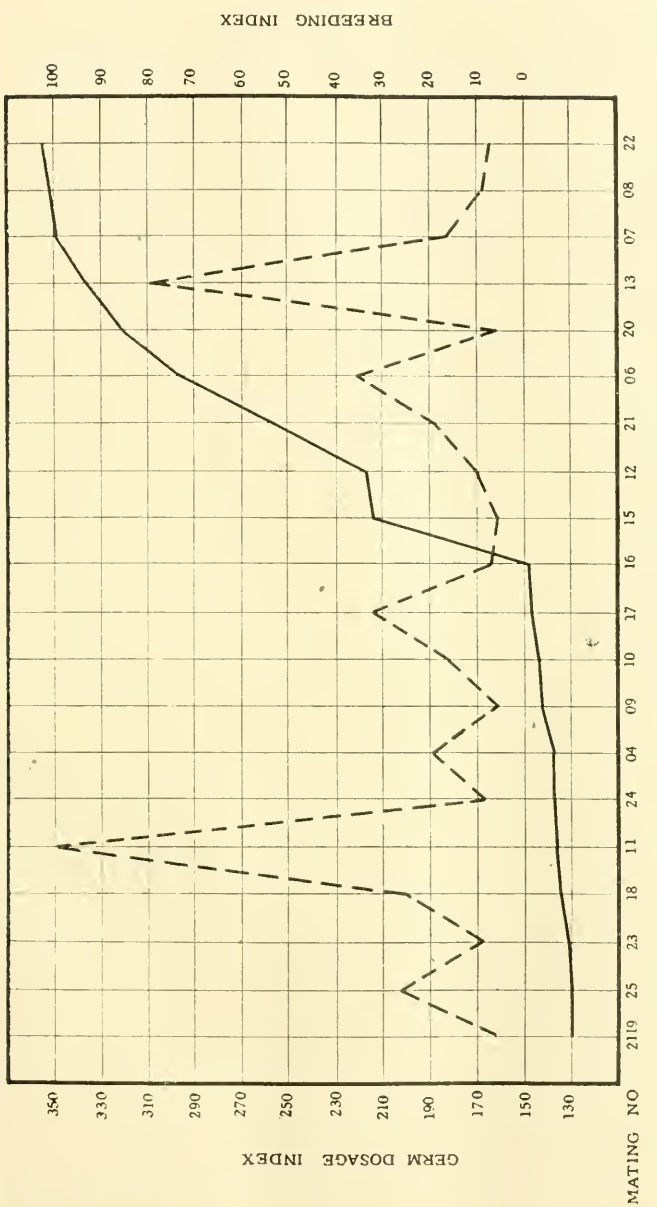

Fig. 1 Diagram showing the relation between total reproductive capacity and total germ dosage index of alcoholized individuals. Solid line, total germ dosage index; dash line, breeding index for the same matings.

Calculating the coefficient of correlation between these two variables, breeding index and germ dosage index, we get

$$r = +0.012 \pm 0.151,$$

a value equal to zero, within the errors of random sampling. It thus appears that there is no relation between the reproductive capacity (as here defined) of the alcoholized birds and the total dosage to which their gametes were subjected in the breeding season of 1915.

IV. DATES OF HATCHING OF F_1 CHICKS

In order to deal critically with certain classes of data to be presented in later sections of the paper it is essential to have in hand the facts in regard to date of hatching of the chicks from treated and control parents. The data on this point are exhibited in table 4. In the last column of this table the distribution is given for time of hatching of the F_1 chicks of the cross Black Hamburg ♂ × Barred Plymouth Rock ♀ hatched from normal, untreated parents in the season of 1913. These chicks are used as controls on the chicks from treated parents in certain later sections of the paper.

The data presented in this table show that the greatest difference between any two lots of birds in mean date of hatching is between the methyl ♂ × untreated ♀ ♀ on the one hand and the ether ♂ × ether ♀ ♀ on the other hand, and amounts to 9.44 days only. The difference is probably significant statistically, but there is no reason to suppose that it is significant biologically. It is well known that differences in the date of hatching may influence certain developmental and physiological characteristics of chickens, and anyone who wishes to do critical experimental work with poultry must guard against comparing in any way lots of chickens having average hatching date differing by large amounts (for example, a month or more). In the present case it is evident that all the chickens in these experiments may for practical purposes be regarded as April hatched. All of the writer's experience with poultry would indicate that no significance is to be attached to such differ-

TABLE 4

Dates of hatching

MONTH AND DAY OF HATCHING	ETHYL ♂ × UNTREATED ♀♀	ETHYL ♂ × ETHYL ♀♀	METHYL ♂ × UNTREATED ♀♀	METHYL ♂ × METHYL ♀♀	ETHER ♂ × UNTREATED ♀♀	ETHER ♂ × ETHER ♀♀	ALL TREATED ♂♂ × UNTREATED ♀♀	ALL TREATED ♂♂ × TREATED ♀♀	1913 NORMAL CONTROL
March 31	12	0	15	6	11	0	38	6	0
April 1	2	11	3	0	1	8	6	19	0
7	0	0	0	0	0	0	0	0	1
8	0	0	0	0	0	0	0	0	1
9	0	0	0	0	0	0	0	0	32
10	0	0	0	0	0	0	0	0	18
12	1	5	7	4	16	0	24	9	0
21	0	0	0	0	0	0	0	0	6
22	0	0	0	0	0	0	0	0	23
23	0	0	0	0	0	0	0	0	11
24	0	0	0	0	0	0	0	0	2
26	27	0	11	0	31	0	69	0	0
27	3	15	0	4	0	22	3	41	0
May 1	0	0	0	0	0	0	0	0	16
2	0	0	0	0	0	0	0	0	18
19	0	0	0	0	0	0	0	0	22
21	0	0	0	0	0	0	0	0	20
27	0	0	0	0	0	0	0	0	6
June 2	0	0	0	0	0	0	0	0	7
3	0	0	0	0	0	0	0	0	12
10	0	0	0	0	0	0	0	0	4
16	0	0	0	0	0	0	0	0	9
Totals.....	45	31	36	14	59	30	140	75	
Mean date of hatching, April	17.71±1.21	15.35±1.44	10.63±1.27	11.14±2.02	16.93±0.91	20.07±1.42	15.49±0.66	16.45±0.94	15.06±0.45[1]
Standard deviation of date of hatching.....	11.99±0.85	11.86±1.02	11.28±0.90	11.19±1.43	10.37±0.64	11.50±1.00	11.55±0.47	12.06±0.66	6.46±0.32[1]

[1] These figures are calculated from chicks hatched before May 1 only. See text p. 270.

ences in mean dates of hatching as appear in table 4 as a possible factor in explaining differences in results obtained.

In the case of the 1913 normal controls in the last column of the table the hatching extended over a longer period of time. But in using these birds as controls on hatching weight, growth, etc., of the alcoholic chicks, only those hatched before May 1 have been used. The mean date of hatching of the April hatched chicks of these 1913 controls is almost identical with the mean dates for the chicks in the alcohol experiments.

V. MORTALITY OF F_1 CHICKS

According to the results of earlier workers in this general field it would be expected that there would be a decidedly higher rate of mortality among the offspring of the alcoholized parents than the normal. The data on this point are exhibited in tables 5 and 6. In these tables the first column gives the total number of chicks tagged with a numbered leg band and put into a brooder. The circumstance that the numbers in this column do not agree with the total numbers of chicks hatched, as given in table 1, arises simply from the fact that not every chicken which hatches is banded. Chicks which are obviously malformed, or lacking in vitality to a degree which makes their death within a few hours certain are not banded. Also entirely normal chickens may not be banded because not wanted for any further purpose. It very often happens that after the available housing space is completely filled with chickens one will carry through further hatches for the purpose of adding more data on the fertility and hatching quality of eggs and for no other purpose. When such chickens hatch they are not banded, but immediately disposed of in another way. Also some entirely normal chicks may fail to be banded by oversight or accident, though this happens only very rarely. The figures in the first column of tables 5 and 6 may be taken as indicating chickens which started life as sound and healthy individuals, so far as appearances indicated. "Chicks missing" recorded in the second column include in the first place those that lost their leg bands through accident and thus could not

TABLE 5

Mortality records of progeny of treated birds

NATURE OF MATINO	CHICKS BANDED			CHICKS DIED UNDER 180 DAYS		PER CENT MORTALITY UNDER 180 DAYS, BOTH SEXES	PER CENT MORTALITY UNDER 180 DAYS		BIRDS IN HEALTHY CONDITION AT 180 DAYS OF AGE	BIRDS PUT IN WINTER QUARTERS	BIRDS DYING AT OVER 180 DAYS
	♂♂	♀♀	Missing, sex not known	♂♂	♀♀		♂♂	♀♀			
Ethyl ♂ × untreated ♀	21	21	3	5	9	33.3	23.8	42.9	28	4	0
Ethyl ♂ × ethyl ♀	14	16	1	0	3	10.0	0	18.7	27	8	0
Totals: Ethyl ♂	35	37	4	5	12	23.6	14.3	32.4	55	12	0
Methyl ♂ × untreated ♀	22	12	2	1	2	8.8	4.5	16.7	31	6	1
Methyl ♂ × methyl ♀	6	6	2	0	0	0	0	0	12	6	1
Totals: Methyl ♂	28	18	4	1	2	6.5	3.6	11.1	43	12	2
Ether ♂ × untreated ♀	22	35	2	8	3	19.3	36.4	8.6	46	7	0
Ether ♂ × ether ♀	10	14	6	1	3	16.7	10.0	21.4	20	8	2
Totals: Ether ♂	32	49	8	9	6	18.5	28.1	12.2	66	15	2
Grand totals: All treated ♂♂ × untreated ♀♀	65	68	7	14	14	21.1	21.5	20.6	105	17	1
Grand totals: All treated ♂♂ × treated ♀♀	30	36	9	1	6	10.6	3.3	16.7	59	22	3
Grand totals: All treated	95	104	16	15	20	17.6	15.8	19.2	164	39	4

be individually identified, and in the second place those that were killed and carried off by predaceous enemies (chiefly crows, hawks, and rats).

The mortality is considered in two groups according to the age at death. In the first group are included all individuals dying before attaining the age of 180 days; in the second group all mortality at higher ages. Finer grouping might of course be made but for present purposes seems unnecessary. Six months covers practically the whole of the life on free range as a chick. To that age all individuals are given every opportunity to live if they are able to do so.

It has not been thought necessary to deal with each individual mating separately in table 5. Instead the matings of a particular class are grouped together. Table 6 gives the mortality, in 1915, of the chicks from three different sets of matings of normal, untreated parents, to serve as controls on the treated matings of table 5. The first set of controls consist of the random sample of 22 matings in which the female was a pure Barred Plymouth Rock. These are the same 22 matings that were used in table 2. The second set of control data includes all F_1 matings, regardless of the breeds crossed, which were made in 1915. The degree of heterozygosity of the chicks from these matings is the same as that of the chicks of treated parents dealt with in table 5. The third set of data for control comparison consists of the mortality results from 22 selected matings of 1915, the basis of the selection being the amount of mortality. The 22 matings included were the ones showing the lowest mortality losses of all the matings of the year.

From these tables we note the following points:

1. Taking the grand total figures from table 5 it appears that the average percentage of mortality during the first six months of life was distinctly lower in the case of chicks, one or both of whose parents were treated, than in the case of any chicks from normal, untreated parents, except as compared with those coming from the selected best matings. These selected best matings are practically identical with the treated matings in respect of average mortality, differing by less than 2 per cent.

TABLE 6

Mortality records of progeny of untreated parents. Control on Table 5

NATURE OF MATING	CHICKS BANDED			CHICKS DIED UNDER 180 DAYS		PER CENT MORTALITY UNDER 180 DAYS, BOTH SEXES	PER CENT MORTALITY UNDER 180 DAYS		BIRDS IN HEALTHY CONDITION AT 180 DAYS OF AGE	BIRDS PUT IN WINTER QUARTERS	BIRDS DYING AT OVER 180 DAYS
	♂♂	♀♀	Missing, sex not known	♂♂	♀♀		♂♂	♀♀			
Random sample matings of 1915, as in table 2	120	148	13	46	75	45.2[1]	38.3	50.7	148	60	10
All F_1 matings of 1915, of untreated ♂♂ × untreated ♀♀	38	27	2	12	12	36.9	31.6	44.4	41	17	1
Best 22 matings of 1915	160	184	24	22	32	15.7	13.7	17.4	290	44	6

[1] One chick of unrecorded sex died under 180 days and is reckoned in this percentage.

In other words, there is no evidence that the ability of the offspring to live was in any way adversely affected by the fact that one or both parents had been systematically alcoholized (or etherized).

2. This result takes on added significance in view of the fact that the chick mortality on the Station poultry plant was, in general, very unusually high in the season of 1915. This came about from the fact that the rearing of the chicks was put in the hands of a man who had had no previous experience in this sort of work. In the process of learning to feed and brood chicks properly he managed to kill an extraordinarily large number. It required a chick of highly vigorous constitution to survive. All weaklings of low vitality and defectives were quickly eliminated by this drastic natural selection. This fact is abundantly evident from the first two lines of table 6.

3. Offspring of the ethyl male showed the highest average percentage chick mortality. The ether male gave a mortality about 5 per cent lower. The offspring of the methyl male exhibited a very low rate of mortality, both absolutely and relatively. The latter fact probably means no more than that his chickens were exceptionally strong, vigorous specimens, totally uninfluenced by the parental treatment. It will be recalled that this same male gave the lowest percentage of mortality in the shell before hatching (cf. table 1). This bears out the general fact that there is a tendency in poultry towards a distinct positive correlation between the prenatal and postnatal mortality.

4. In every group the offspring of treated females (treated ♂) showed a lower average percentage of chick mortality than did the offspring of normal untreated females mated to the same males. Proportionally about half as many chicks of the former sort died as of the latter. Again this parallels the facts regarding prenatal mortality brought out in table 1.

5. Out of the 39 offspring of treated parents which went into winter quarters only 4, or approximately 10 per cent died before February 1, 1916. This is a smaller adult mortality than is shown by any of the control lots except the second.

6. The absolute chick mortality tends to be higher among the females than among the males in general. This is true in four out of the six basic groups in table 5. The two exceptions are, first, methyl ♂ methyl ♀, in which group there was no chick mortality whatever, and second, ether ♂ × untreated ♀, where 8 ♂♂ to 3 ♀♀ died. In two of the control groups of table 6 more female chicks than male died. In the other group absolute mortality of the two sexes was equal.

7. Turning to the figures for the relative mortality we find that in only one of the groups in which there was some mortality did a higher percentage of males than of females die. This was in the group ether ♂ × untreated ♀. The considerable excess of male deaths in this group, taken in connection with the generally low absolute mortality, was sufficient to bring the percentage mortality of males slightly, but not significantly, greater than that of females in the general group treated ♂ × untreated ♀♀. There seems to be no warrant for supposing that the generally greater proportional mortality of females shown in table 5 is in any way due to the alcoholization, since precisely the same thing is shown in all the control series of table 6. Taking grand totals the relative mortality of females is 3.4 per cent higher than that of the males among the offspring of treated males, while it is 3.7 per cent higher in the best 22 matings of 1915. Since the last mentioned series shows a general chick mortality rate nearest to that of the alcohol series as a whole, it would seem to be the fairest control series for such a relative comparison.

Taking all the evidence in the case into account, it admits of no doubt that the probability that a chick on the Maine Station's poultry range in 1915 would survive to maturity was not diminished, but, on the contrary, was in general substantially increased if that chick's parents had both been subjected to a daily dosage of alcohol for from four to seven months before it was hatched. Since the chicks from treated parents were indiscriminately mixed with those from normal parents in housing, yarding, feeding, watering, etc., the fact that the former sort of chicks showed a lower mortality than the latter sort can

not be attributed to differential treatment after hatching. This result furthermore can not reasonably be supposed to be due to any superiority in innate constitutional vigor in the parent stock used in the case of the alcoholics. Such a supposition is definitely negatived by the pedigree data presented in I, and also by the method followed of using sisters as controls.

It will be noted that the results in respect of postnatal mortality are exactly in accord with those already noted for prenatal mortality (p. 261). The progeny of treated parents have a lower rate of mortality than the progeny of untreated. The most probable explanation of all these results appears to be the one which has already been suggested in dealing with prenatal mortality, namely that the alcohol (or ether) acts as a selective agent upon the germ cells, preventing the formation of zygotes by any except the strongest and most resistant gametes.

VI. THE SEX RATIO IN THE F_1 PROGENY

It has been claimed at various times and by various persons that the general metabolic condition of the parents at the time of conception is a factor in sex determination, or at least has an influence on the sex ratio. Particularly convincing evidence that this is the case in various species of rotifers has been afforded by the recent papers of Whitney (43). Russo's (33) attempt to demonstrate that in mammals the nutritive condition of the germ cells is a limiting factor in sex determination was not so successful, Basile (1) and Punnett (32) failing to confirm his results. It is of importance in any experiment like the present, whatever one's *a priori* opinions about the probable outcome, to examine with some care the sex ratios obtained, in order to see whether by any chance the parental treatment has influenced them in any way. In particular does such an examination appear desirable in view of the recent statement by Stockard and Papanicolaou (38, p. 166), in discussing the different results in the two sexes of the progeny of alcoholized guinea pigs. They say:

The only plausible way to account for the origin of this difference is to assume that the female-producing spermatozoa were more modified by the treatment than the male-producing spermatozoa. Whether such an increased modification is due to the presence of a greater mass of chromatin to be injured in the one case than in the other or to a difference in response on the part of the two heteromorphic sex chromosomes it is impossible to state. The difference, however, is a fact!

A part of the pertinent data on this point in our fowls has been presented in table 5. The remainder is exhibited in tables 7 and 8. Table 7 gives the sex of the progeny of treated parents, by individual matings. The results by grouped matings according to treatment are shown in table 5.

Table 8 gives the sex ratio statistics for the series of matings of normal Black Hamburg ♂♂ × Barred Plymouth Rock ♀♀ made in 1913. All the parents in this series were untreated normal individuals. Again the data are arranged by individual matings in order that an appreciation of the amount of variation in the sex ratio may be obtained.

It is obvious from these tables that the male sex ratio varies to a considerable degree, especially in the treated series. It is doubtful, however, whether the matings in the treated series are really significantly more variable in this respect, having regard to probable errors. Giving each mating equal weight, that is not weighting with fertility, we get the following constants:

Treated ♂ × normal ♀♀ : Unweighted mean ♂ sex ratio = 48.4±5.3%
Treated ♂ × normal ♀♀ : Unweighted standard deviation = 26.3±3.8%
Treated ♂ × treated ♀♀ : Unweighted mean ♂ sex ratio = 47.0±7.1%
Treated ♂ × treated ♀♀ : Unweighted standard deviation = 27.7±5.0%
Normal ♂♂ × normal ♀♀ : Unweighted mean ♂ sex ratio = 53.2±2.4%
Normal ♂♂ × normal ♀♀ : Unweighted standard deviation = 16.0±1.7%

Turning to the weighted means and their probable errors given in tables 7 and 8 we derive the following set of differences with their probable errors:

Weighted mean ♂ sex ratio differences

(Normal ♂♂ × normal ♀♀) − (treated ♂ × normal ♀♀) = 1.1±3.6%
(Normal ♂♂ × normal ♀♀) − (treated ♂ × treated ♀♀) = 4.5±4.7%
(Treated ♂♂ × normal ♀♀) − (treated ♂ × treated ♀♀) = 3.4±5.0%

TABLE 7

Showing the sex ratio in matings of treated black Hamburg ♁ ♁ A. with untreated B. P. R. ♂♂

MATING NUMBERS	TOTAL CHICKS	SEX UNKNOWN	♂♂	♀♀	$\frac{100\ ♂♂}{♂♂ + ♀♀}$
2116	3	2	0	1	0
2117	14	1	5	8	38.5
2118	21	0	14	7	66.7
2119	7	0	2	5	28.6
2123	5	0	4	1	80.0
2124	5	0	5	0	100.0
2125	26	2	13	11	54.2
2104	17	1	6	10	37.5
2109	5	0	3	2	60.0
2110	11	0	3	8	27.3
2111	26	1	10	15	40.0
Totals and mean.....	140	7	65	68	48.9±2.9[1]
B. with treated B. P. R. ♀♀					
2112	11	0	4	7	36.4
2113	19	0	10	9	52.6
2115	1	1	0	0	
2120	1	0	1	0	100.0
2121	12	1	5	6	45.5
2106	18	2	6	10	37.5
2107	2	1	0	1	0
2108	10	3	4	3	57.1
Totals and mean	74	8	30	36	45.5±4.1[1]

[1] These probable errors are calculated by the usual formula for the probable error of a percentage: $0.6744898 \ldots \sqrt{\frac{p \cdot q}{N}}$, where p denotes the percentage of which the probable error is desired, $q = 100 - p$, and N = the total number of observations on which p is based.

Evidently these figures give no ground for asserting that the relative proportions of the sexes produced are significantly different in the alcoholic and normal control series. If the treatment has had an influence on this character it has been so slight as not to be statistically discernible in samples of the size here dealt with.

VII. HATCHING WEIGHT OF F_1 PROGENY

Cole and Davis (4) found a reduction in the birth weight of rabbits when the father was alcoholic. Cole and Bachhuber (3, p. 25) report a reduction in birth weight of guinea pigs from a lead poisoned father. This result has been confirmed and extended by Weller (42, p. 292) who reports an average reduc-

TABLE 8

Showing the sex ratio in matings of untreated black Hamburg ♂ × B. P. R. ♀

MATING NUMBERS	TOTAL CHICKS	SEX UNKNOWN	♂♂	♀♀	$\frac{100\ ♂♂}{♂♂ + ♀♀}$
1660	11		8	3	72.7
1661	22	1	10	11	47.6
1662	12	1	6	5	54.5
1663	22		7	15	31.8
1664	9		3	6	33.3
1665	2		1	1	50.0
1666	6		3	3	50.0
1667	29		16	13	55.2
1668	22	2	9	11	45.0
1669	19		7	12	36.8
1675	6		4	2	66.7
1676	2		2	0	100.0
1677	11		7	4	63.6
1678	2		1	1	50.0
1679	11		8	3	72.7
1680	13		8	5	61.5
1681	3		1	2	33.3
1682	6		3	3	50.0
1891	17	1	7	9	43.7
1905	27	5	10	13	45.5
Totals and mean.....	252	10	121	121	50.0±2.2

tion of about 20 per cent in birth weight of guinea pigs from lead poisoned parents. Stockard has given no figures for mean birth weight in his experiments, but the inference from all of his data, particularly that relating to the proportion of dwarfed young, is that had birth weights been systematically recorded in his experiments, the mean would have been distinctly lower in the offspring of treated parents than in those from normal parents.

The hatching weight of a bird is not directly comparable with the birth weight of a mammal physiologically. Probably the chief factor in determining the weight of a chick at hatching is the size of the egg from which it hatched. There is a high positive correlation between the initial weight of the whole egg and the body weight of the chick which hatches from it. Furthermore, external conditions during incubation, especially with reference to moisture, influence the weight at hatching. Finally the innate constitutional vigor of the embryo, reflected in its ability to grow, is a factor in the determination of the hatching weight. In experimental work which involves hatching weight as a datum, the differential effects of egg size and conditions of incubation, can be practically eliminated provided the experiments are carefully and critically planned and carried out. The innate factor then comes forth in full importance. Such conditions are believed to have been realized in the present work.

The frequency distributions for hatching weight are given in table 9, and the variation constants deduced from them in table 10. The material in the alcohol series is treated in the two groups "Treated ♂ × Untreated ♀ ♀" and "Treated ♂ × Treated ♀ ♀" rather than by individual matings, or by substance. An examination of the hatching weight distributions by substance (ethyl alcohol, methyl alcohol, and ether) shows that there is no essential difference between the different groups. On this account there seems no reason to take the space here which would be required to show all the separate distributions in detail. As a control for these and all other growth data we have the 1913 normals of the same cross, from the same stock. In order that no possible error may creep in from differences in date of hatching, use has been made here, and in all other growth data in this paper, of the normal cross-breds hatching in the month of April, 1913, only. A reference to table 4 will show that the mean dates of hatching for the control chicks used in this study and the chick of the treated series are almost exactly identical.

TABLE 9

Frequency distributions for hatching weight

HATCHING WEIGHT IN GRAMS	TREATED ♂ × UNTREATED ♀♀		TREATED ♂ × TREATED ♀♀		1913 CONTROLS. UNTREATED ♂♂ × UNTREATED ♀♀. (APRIL HATCHED)	
	♂	♀	♂	♀	♂	♀
24.5–25.4					1	
25.5–26.4						
26.5–27.4					1	
27.5–28.4						1
28.5–29.4	3				1	
29.5–30.4	1	4			1	1
30.5–31.4	10	7	2		3	6
31.5–32.4	5	5			2	3
32.5–33.4	8	7	2	2	7	8
33.5–34.4	2	8	5	2	3	3
34.5–35.4	10	12	3	9	7	13
35.5–36.4	5	4	3	5	2	6
36.5–37.4	4	5	3	4	4	6
37.5–38.4	2	5	4	2	1	6
38.5–39.4	10	3		5	2	
39.5–40.4		1	3	2		
40.5–41.4	4	7	2	2	2	1
41.5–42.4	1			2		1
42.5–43.4			2	1		
43.5–44.4						
44.5–45.4						
45.5–46.4						
46.5–47.4			1			
Total.....	65	68	30	36	37	55

TABLE 10

Variation constants for hatching weight

CONSTANT	TREATED ♂ × UNTREATED ♀♀		TREATED ♂ × TREATED ♀♀		1913 CONTROLS. UNTREATED ♂♂ × UNTREATED ♀♀	
	♂	♀	♂	♀	♂	♀
Mean.....	34.91±0.29	35.04±0.26	36.97±0.45	37.17±0.29	34.24±0.37	34.73±0.24
Standard deviation.	3.42±0.20	3.17±0.18	3.64±0.32	2.58±0.20	3.32±0.26	2.67±0.17
Coefficient of variation.....	9.78±0.58	9.03±0.53	9.83±0.87	6.93±0.55	9.69±0.77	7.69±0.50

From table 10 we derive the following series of differences and their probable errors in respect to mean hatching weight of the chicks in the different series:

Male chicks

(Treated ♂ × untreated ♀♀) − (controls) = +0.67±0.47 gram.
(Treated ♂ × treated ♀♀) − (controls) = +2.73±0.58 gram. (× 4.7)
(Treated ♂ × treated ♀♀) − (treated ♂ × untreated ♀♀) = +2.06±0.54 (× 3.8)

Female chicks

(Treated ♂ × untreated ♀♀) − (controls) = +0.31±0.35 gram.
(Treated ♂ × treated ♀♀) − (controls) = +2.44±0.38 gram. (× 6.4)
(Treated ♂ × treated ♀♀) − (treated ♂ × untreated ♀♀) = +2.13±0.39 (× 5.5)

Sex differences

Treated ♂ × untreated ♀♀ series: ♀ − ♂ = 0.13±0.39 gram.
Treated ♂ × treated ♀♀ series: ♀ − ♂ = 0.20±0.54 gram.
Control series: ♀ − ♂ = 0.49±0.44 gram.

From these figures the following conclusions appear to be warranted:

1. In the present series of experiments there is no significant difference in mean hatching weight between the offspring of treated males and the offspring of normal untreated control males when both are mated to normal untreated females. The slight differences which do appear are of the same order of magnitude as their probable errors.

2. Both the male and the female offspring of matings in which both parents were treated have a larger mean hatching weight (i.e., are heavier when hatched) than the offspring of either completely normal control matings, or of matings in which the father only is treated. The differences here are relatively large and are statistically significant in comparison with their probable errors. The figures in parentheses following the difference lines give the ratio Diff./P. E. diff. These figures range from 3.8 to 6.4. From the table given by Pearl and Miner (24, p. 88) it appears that the odds against the fortuitous occurrence of deviations as great as or greater than these range from about 95 to 1 to about 20,000 to 1. They may fairly be considered real and significant differences.

3. The mean hatching weights of the females are in all three series slightly greater than those of the males. The differences, however, are entirely insignificant in comparison with their probable errors.

Turning to the consideration of relative variability in hatching weight, as measured by the coefficient of variation, it appears that the males are more variable than the females in all three series. The differences, however, are not very large in proportion to their probable errors, and can be regarded only as somewhat doubtfully significant.

There appears to be no evidence that the relative variability in respect of hatching weight has been in any way affected by the alcohol treatment.

VIII. GROWTH OF THE F_1 PROGENY

Growth, as measured by increase in body weight, is universally recognized by physiologists and by practical animal husbandmen as one of the most valuable indices of innate constitutional vigor and vitality which it is possible to obtain. On this account it was thought to be of first-class importance to study the growth of the offspring from alcoholized as compared with untreated parents.

The frequency distributions giving the raw data on the growth of the animals in these experiments are exhibited as Appendix Tables I to XV inclusive. The constants deduced from these distributions are given in tables 11, 12, and 13. Regarding the collection of the growth data it may be said that weighings were made at regular intervals according to a fixed schedule. For purposes of analysis weighings for ages differing by but few days were grouped, and regarded as concentrated at the central age of the group. This amounts essentially to a first smooth of the material. It is not our intention in the present paper to enter upon any discussion of general problems of growth. It is desired here merely to make a comparison between the offspring of alcoholized and those of non-alcoholized parents.

It will be noted by comparison of tables 11 to 13 that the centered ages for which mean body weights are given are in

TABLE 11

Variation constants for growth in body weight of offspring in the treated ♂ × untreated ♀ ♀ series

AGE IN DAYS	MEAN (IN GRAMS)		STANDARD DEVIATION (IN GRAMS)		COEFFICIENT OF VARIATION	
	♂	♀	♂	♀	♂	♀
0 (Hatching)	34.91± 0.29	35.04± 0.26	3.42± 0.20	3.17± 0.18	9.78±0.58	9.03±0.53
5.5	40.94± 0.45	41.58± 0.47	5.39± 0.32	5.31± 0.33	13.18±0.80	12.77±0.81
12.5	55.50± 0.83	55.94± 0.79	9.00± 0.58	8.68± 0.56	16.22±1.08	15.51±1.02
19.5	84.06± 1.53	83.07± 1.48	16.18± 1.08	16.40± 1.05	19.25±1.33	19.75±1.31
33.5	181.75± 3.22	174.13± 3.33	34.07± 2.28	36.25± 2.35	18.74±1.29	20.82±1.41
47.5	331.17± 6.31	309.50± 5.80	64.79± 4.46	62.00± 4.10	19.56±1.40	20.03±1.38
68.5	621.67± 8.27	562.27± 9.45	83.15± 5.85	96.09± 6.68	13.38±0.96	17.08±1.22
89.5	981.36±11.71	852.38±12.31	124.02± 8.28	131.65± 8.71	12.64±0.86	15.45±1.05
110.5	1398.03±15.68	1125.46±13.74	166.05±11.09	146.87± 9.71	11.88±0.80	13.05±0.88
138.5	1867.50±18.36	1457.19±13.06	192.46±12.98	139.61± 9.23	10.31±0.70	9.58±0.64
166.5	2263.79±23.68	1682.83±16.58	245.75±16.74	175.50±11.72	10.86±0.75	10.43±0.70
194.5	2570.55±36.48	1956.64±31.87	235.72±25.79	176.80±22.54	9.17±1.01	9.04±1.16
204.5	2749.50±68.84	2009.50±35.13	250.00±48.68	116.48±24.84	9.09±1.79	5.80±1.24
290.5		2030.06±27.57		122.63±19.50		6.04±0.96

TABLE 12

Variation constants for growth in body weight of offspring in the treated ♂ × treated ♀♀ series

AGE IN DAYS	MEAN (IN GRAMS)		STANDARD DEVIATION (IN GRAMS)		COEFFICIENT OF VARIATION	
	♂	♀	♂	♀	♂	♀
0 (Hatching)	36.97± 0.45	37.17± 0.29	3.64± 0.32	2.58± 0.20	9.83±0.87	6.93±0.55
5.5	44.91± 0.59	42.68± 0.55	4.67± 0.41	4.64± 0.39	10.41±0.93	10.88±0.91
12.5	57.84± 1.02	54.60± 0.72	8.16± 0.72	5.96± 0.51	14.10±1.27	10.92±0.95
19.5	89.41± 1.96	82.69± 1.86	15.61± 1.38	14.89± 1.32	17.46±1.59	18.00±1.65
33.5	194.13± 5.92	176.64± 3.43	45.57± 4.18	26.88± 2.42	23.48±2.27	15.22±1.40
47.5	358.07± 6.92	317.65± 5.34	54.32± 4.90	41.14± 3.78	15.17±1.40	12.95±1.21
68.5	663.79±12.70	587.43± 8.93	99.67± 8.98	71.27± 6.31	15.02±1.38	12.13±1.09
89.5	1026.42±22.79	867.83±12.67	172.31±16.12	102.86± 8.96	16.79±1.61	11.85±1.05
110.5	1417.09±26.12	1147.83±14.17	201.19±18.47	115.10±10.02	14.20±1.33	11.03±0.88
138.5	1870.93±32.53	1469.50±17.17	255.22±23.00	139.40±12.14	13.64±1.25	9.49±0.83
166.5	2315.02±33.55	1702.83±20.23	267.90±23.73	164.28±14.31	11.57±1.04	9.65±0.85
194.5	2724.50±33.37	1982.83±44.98	171.39±23.60	258.31±31.81	6.29±0.87	13.03±1.63
204.5	2889.50±67.16	2024.50±46.82	222.64±47.49	196.32±33.10	7.71±1.65	9.70±1.65
290.5		2097.23±54.14		266.21±38.28		12.69±1.85

TABLE 13

Variation constants for growth in body weight of offspring in the normal control untreated ♂♂ × untreated ♀♀ series

AGE IN DAYS	MEAN (IN GRAMS)		STANDARD DEVIATION (IN GRAMS)		COEFFICIENT OF VARIATION	
	♂	♀	♂	♀	♂	♀
0 (Hatching)	34.24± 0.37	34.73± 0.24	3.32± 0.26	2.67± 0.17	9.69±0.77	7.69±0.50
2.0	32.33± 0.45	32.23± 0.28	2.30± 0.32	1.94± 0.20	7.12±0.99	6.03±0.62
5.5	41.70± 0.86	40.79± 0.74	5.71± 0.61	5.77± 0.52	13.70±1.49	14.15±1.30
9.0	51.29± 1.09	54.95± 0.89	6.06± 0.77	6.17± 0.63	11.81±1.53	11.23±1.16
12.5	64.50± 1.54	62.54± 1.34	10.19± 1.09	10.54± 0.95	15.79±1.73	16.85±1.56
16.0	67.00± 1.75	71.09± 1.14	9.71± 1.24	7.94± 0.81	14.50±1.89	11.17±1.15
19.5	98.32± 2.34	91.29± 2.20	15.14± 1.66	17.30± 1.56	15.40±1.72	18.95±1.79
23.0	107.71± 3.54	108.14± 2.15	19.66± 2.51	14.93± 1.52	18.26±2.40	13.80±1.43
26.5	137.53± 2.38	128.25± 1.56	15.40± 1.68	12.24± 1.10	11.20±1.24	9.55±0.87
37.0	239.05± 5.20	238.14± 3.75	25.55± 3.67	26.05± 2.65	10.69±1.56	10.94±1.13
40.5	235.55± 6.53	223.30± 5.40	42.19± 4.61	40.03± 3.82	17.91±2.02	17.93±1.76
53.0	416.40± 8.93	386.39± 4.78	71.26± 6.31	47.53± 3.38	17.11±1.56	12.30±0.89
67.0	645.09±11.71	584.73± 7.49	101.24± 8.28	73.66± 5.30	15.69±1.32	12.60±0.92
77.5	835.21±17.57	739.08±10.97	97.46±12.42	79.69± 7.76	11.67±1.51	10.78±1.06
95.0	1081.17±19.37	926.77± 8.88	157.31±13.70	87.34± 6.28	14.55±1.29	9.42±0.68
119.5	1405.93±27.10	1214.70±14.51	237.74±19.17	153.68±10.26	16.91±1.40	12.65±0.86
147.5	1860.61±22.45	1533.32±14.20	199.69±15.87	150.35±10.04	10.73±0.86	9.81±0.66
165.0	2131.64±27.48	1747.50±19.01	186.71±19.43	199.28±13.44	8.76±0.92	11.40±0.78
174.5		1886.50±26.73		198.11±18.90		10.50±1.01
209.5	2463.07±22.33		195.90±15.79		7.95±0.65	
267.0		1937.00±35.25		209.04±24.92		10.79±1.30
286.0		2020.50±29.49		218.58±20.85		10.82±1.04

many cases not the same for the treated and the untreated series. On this account as well as others a comparison between the different series in respect to growth can best be made by graphical means. To this end figures 2 and 3 have been prepared. It will be noted from these figures that the weighings were carried into adult life in the case of both sexes. They stop somewhat earlier in the case of the males than the females owing to considerations of space on the plant. It scarcely needs to be said that the utmost care was used to ensure accuracy in this growth work, and to keep the surrounding conditions of housing, feed, etc., in every respect favorable to normal growth. The latter point is, of course, a very important one for critical results. The favorableness or unfavorableness for animal growth of the general environmental conditions, the season,' etc., is not a thing which can be exactly measured, but, it may be judged by one experienced in the husbandry of the particular animals dealt with. On such general observational grounds one would have judged that the season and general environmental conditions surrounding the chickens in 1913, the year in which the control chickens *ex* untreated ♂ ♂ × untreated ♀ ♀ were grown, were more favorable to growth than in 1915, the year in which the offspring of alcoholized parents were grown. If such was in fact the case, and my observations lead me to believe that it was, the results obtained, as will presently appear, take on added significance.

It should be particularly noted that no question of artificial selection can enter as a factor in influencing the growth data for the simple reason that no such selection was practiced. Each and every individual chicken was regularly weighed as long as it lived or until it reached maturity and the weighing records came to an end for the season. As the mortality was small it practically means that there was no selection at all between hatching and maturity. In the case of weighings of females at ages over 210 days there has been a selection. The individuals at these higher ages are the ones kept over for winter egg production and to be used as breeders the next year. Great care was taken, however, to ensure that the samples chosen

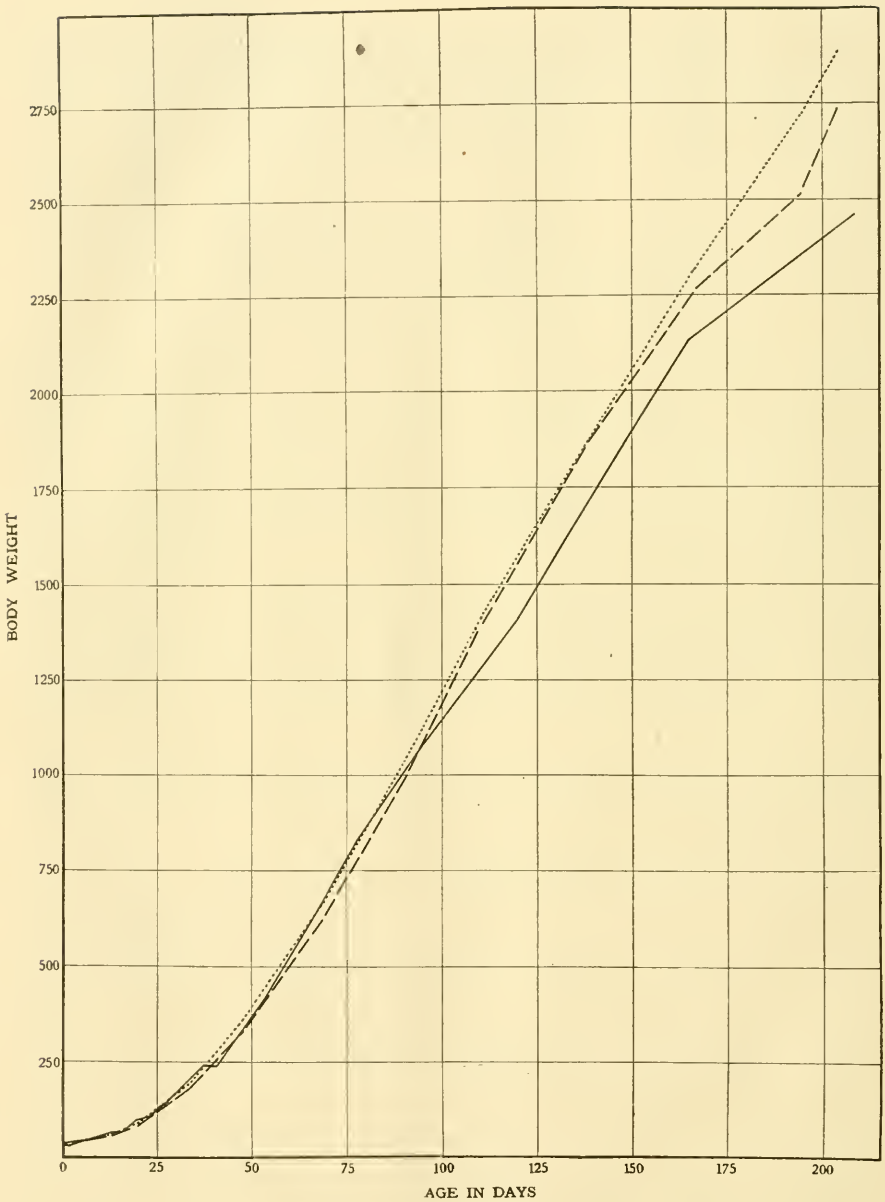

Fig. 2 Diagram showing the growth in body weight of the male offspring of alcoholized parents as compared with the male offspring of untreated parents. Solid line, offspring of untreated ♂♂ × untreated ♀♀; dash line, offspring of treated ♂♂ × untreated ♀♀; dot line, offspring of treated ♂♂ × treated ♀♀.

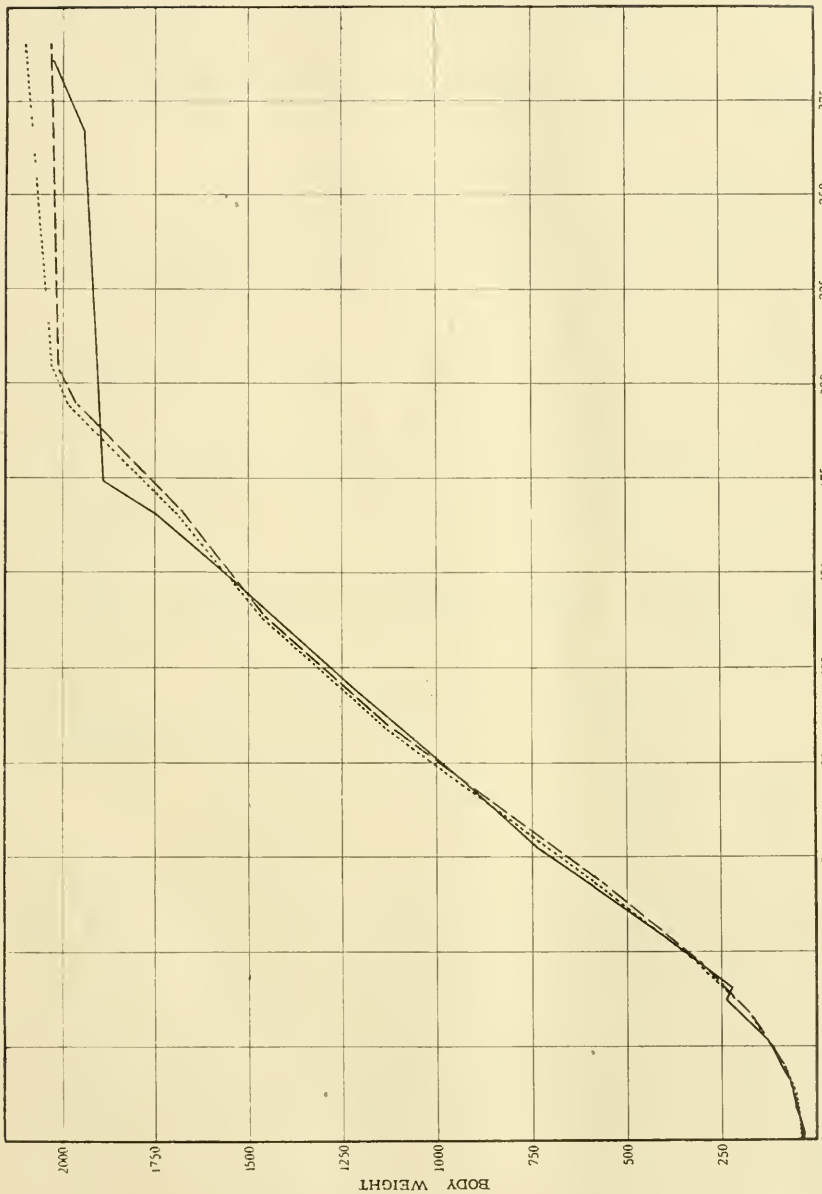

Fig. 3 Diagram showing the growth in body weight of the female offspring of alcoholized parents as compared with the female offspring of untreated parents. Significance of lines as in figure 2.

should be random ones and there is every reason to believe that they were. If anyone, however, hesitates to accept them as such or feels that a greater accuracy necessarily inheres in unsampled material he should confine his consideration of the growth results to ages under 200 days.

From the data in tables 11 to 13 and figures 2 and 3 the following points appear to be well established:

1. The offspring of alcoholized parents, whatever the nature of the mating, showed a higher mean adult body weight than offspring of untreated parents of the same breeds mated in the same way. This is true of both sexes.

2. In the case of the male chickens there was no substantial difference in the rate of growth in the three lots until after an age of about 100 days was passed. From that point on the male offspring of treated ♂♂ × untreated and treated ♀♀ grew at a more rapid rate than the controls. The differences in mean body weight for a given age became increasingly large as the age advanced. At 200 days of age we have, by interpolation on the curves, the following set of comparative mean body weights.

Comparative mean body weights at 200 days of age

	Absolute weight	*Relative weight*
Males *ex* untreated ♂♂ × untreated ♀♀	2392.32 gram	100
Males *ex* treated ♂♂ × untreated ♀♀	2668.97 gram	112
Males *ex* treated ♂♂ × treated ♀♀	2815.25 gram	118

3. In the case of the female chickens there was no substantial difference in the rate of growth in the three lots until after an age of about 150 days was passed. During the next 25 days the controls grew faster than the chicks from treated parents. At and after 200 days of age, however, the offspring of treated parents (one and both) showed a higher mean body weight than the controls. We have the following set of comparative mean body weights at 250 days of age, obtained by interpolation on the curves.

Comparative mean body weights at 250 days of age

	Absolute weight	*Relative weight*
Females *ex* untreated ♂♂ × untreated ♀♀	1927.72	100
Females *ex* treated ♂♂ × untreated ♀♀	2020.38	105
Females *ex* treated ♂♂ × treated ♀♀	2062.98	107

4. At all ages in the case of the male chicks, and in all ages but two (12.5 and 19.5 days) in the case of the female chicks, the mean body weight of the offspring having both parents alcoholic was higher than that of the offspring having one parent only, the father, alcoholic. The differences are, for the most part, insignificant in comparison with their probable errors, but the uniformity with which the dotted curves maintain their superiority over the dash curves is noteworthy.

5. There are no distinctive differences in relative variability between the three different lots of chicks. In general the relative variability tends to diminish after an age of about 30 days is past.

The evidence derived from a study of the growth of the chickens in this experiment lends no support to the view that parental alcoholism reduces the vitality of the offspring or induces degeneracy. On the contrary the data plainly indicate that the offspring of alcoholized parents are in some degree superior in vigor and vitality to those from untreated parents.

IX. DEFORMITIES IN THE F_1 PROGENY

One of the most striking features of Stockard's results on the alcoholization of guinea pigs is that a considerable percentage of the progeny of treated parents exhibit gross malformations of various organs, particularly the eyes. In the present experiments with poultry, nothing of this sort has made its appearance. In breeding poultry on an extensive scale one always gets from perfectly normal parents a certain small number of deformed crippled and weak chickens at the time of hatching. The practical poultryman classes these together as 'cripples.' They apparently are caused primarily by unfavorable conditions during the incubation, and secondarily by deleterious influences acting upon the mother at the time the eggs which are to give

rise to them are produced. One may put the matter in this way: under the conditions which prevail on a large poultry breeding plant it is normal for a certain small percentage of chicks which are abnormal at hatching to be produced. The proportion of such abnormal chicks produced in the breeding season of 1915 from alcoholized parents was no greater than the number produced from untreated parents. In actual fact there was exactly one chicken out of 234 hatched from alcoholized parents in 1915 that was too weak to band, and was in consequence killed at the time of hatching.[5] None was deformed. Out of 1527 chicks from untreated parents 16, or 1.0 per cent were weak or deformed or both.

X. MENDELIAN CHARACTERS IN THE F_1 PROGENY

It is not the intention at this time to enter upon an extended account of the inheritance of characters in the cross Black Hamburg × Barred Plymouth Rock. We have studied this cross for a number of years and have a great mass of material regarding it, based of course upon non-alcoholic birds. This will no doubt be published at some later date. The only point which it is desired to bring out here is that the phenomena of inheritance, in all of a considerable series of characters, are absolutely identical in the F_1 progeny from alcoholic parents and that from untreated parents of the same breeds.

To demonstrate this fact we may consider the data for a few of the characters studied, taking first color pattern. The regular rule of inheritance of color pattern in the F_1 generation of this cross is as follows:

B. H. ♂ (self black) × B. P. R. ♀ (barrred) = Barred ♂♂ + non-barred ♀♀ (self black)

[5] This statement may appear difficult to reconcile with statistics given in tables 1 and 5. It is shown in table 1 that 234 chicks hatched, whereas according to table 5, but 215 were banded. What happened was that, in order to get more extensive statistics on the hatching quality of the eggs, another hatch was brought off on May 12. This produced 18 normal chicks, which were not banded, but immediately discarded. These chicks hence appear in the hatching records, but not in the subsequent data. We have 215 normal banded chicks + 18 normal, but unbanded chicks + 1 abnormal = 234 hatched.

The data from the alcoholic matings of 1915 are as follows:

	Barred	*Non-barred (self black)*
Males	95	0
Females	0	104
Sex not known	8	8

The normality of these results is beyond cavil.

The results regarding comb form are equally clear. The Black Hamburg is a rose-combed breed, the Barred Rock a single-combed. Rose × single normally gives rose in F_1. Out of 215 chicks from the alcohol matings 215, or all, were rose-combed.

Similar data might be given for various other characters. They all are the same, however, in principle, simply showing that the normal regular course of Mendelian inheritance has in no wise been altered or interfered with by the alcoholization of the parents.

XI. DISCUSSION OF RESULTS

Before attempting to discuss any interpretation of the meaning of our results it is first desirable to do something in the way of summarizing them so that a clear and definite picture of the net result may emerge from the mass of statistical data presented in the preceding pages. Such a summary is given in table 14. The casual reader and the hostile critic are strongly urged, however, not to regard table 14 as the only evidence for the conclusions reached. This table aims only to summarize fairly the net results of the detailed statistical evidence in the body of the paper.

The plan of this summary table is as follows. The superior result is printed in bold faced type. In the last column of each table a plus sign denotes that, with reference to the particular character discussed, the progeny of the alcoholists have been favorably affected; a minus sign that they have been unfavorably affected as compared with untreated controls. A zero indicates that no effect of the treatment, one way or the other, has been detected.

We see from this table that *out of 12 different characters for which we have exact quantitative data, the offspring of treated par-*

TABLE 14

Showing in summary form the effect of continued administration of alcohol (ethyl and methyl) and ether, by the inhalation method, upon the progeny

CHARACTER STUDIED	TREATED ♂♂ × UNTREATED ♀♀	TREATED ♂♂ × TREATED ♀♀	ALL TREATED PARENTS	UNTREATED CONTROLS	NET RESULT ON ALCOHOL OFFSPRING
1. Mean germ dosage index..	137.8	299.0	210.35	0	
2. Percentage of infertile eggs (i.e., in which no embryos were formed)..	25.2	59.2	41.7	**25.3**	−
3a. Percentage of embryos dying in shell.....	36.6	**26.9**	33.3	42.2	+
3b. Percentage of fertile eggs which hatched.....	63.0	**12.3**	66.7	57.8	+
4. Percentage of all eggs which hatched.....	47.1	29.4	38.6	**44.4**	−
5. Percentage mortality under 180 days of age.....	21.1	**10.6**	17.6	36.9	+
6. Percentage mortality over 180 days of age.....	**5.9**	13.6	10.3	15.3	+
7. Sex ratio: per cent ♂♂...	48.9	45.5	47.7	50.0	0
8. Mean hatching weight per bird, males.....	34.91	**36.97**		34.24	+
9. Mean hatching weight per bird females.....	35.04	**37.17**		34.73	+
10. Mean adult weight per bird, males.....	2669	2815		2392	+
11. Mean adult weight per bird, females.....	2020	**2063**		1928	+
12. Percentage of weak and deformed chicks.....	0.7	**0**	0.4	1.0	+
13. Abnormalities of Mendelian inheritance.....	0	0	0	0	0

ents taken as a group are superior to the offspring of untreated parents in 8 characters. The offspring of untreated parents are superior to those of the alcoholists in respect of but two characters, and these are characters which are quite highly correlated with each other. Finally with respect to two character groups there is no difference between the alcoholists and the non-alcoholists. The character groups which have been dealt with in this study, and for which definite quantitative data are herein presented, seem to cover a much wider range of physio-

logical and genetic factors and phenomena than has ever been included, or even approached, in any previous study regarding the effects of parental alcoholism upon the progeny. They have the further advantage of being characters which are measurable (either statistically or otherwise) and on that account greatly reduce, if they do not entirely eliminate the possibility of personal bias or prejudice influencing the results. For example, to take but a single instance, the weighings for growth records were made by my assistant Dr. Maynie R. Curtis, in the case of all progeny of alcoholic parents, and for all ages except hatching where the writer himself did the weighing. The weights made in the field by Dr. Curtis were posted into the permanent record books by another assistant, Miss Hazel F. Mariner, and were reduced to the form of frequency distributions some six months later by the staff computer of the laboratory, Mr. John Rice Miner. Not until after all the constants for these alcohol distributions had been completely worked out were the control data from the untreated matings of 1913, where all the weighings were made by the writer, put into the form of frequency distributions. No one of these workers could possibly have known, at any stage in this process before the final one, what the net result in respect to growth was going to be. Similar considerations obtain in regard to the other characters.

The mutual accordance of the results from characters involving such a manifold range of physiological factors is striking. This fact in considerable degree offsets the fact that as yet our series of experimental animals is statistically small, leading to such large probable errors that the individual differences are not in every case significant in comparison with their probable errors. The experiments are of course being continued and expanded, and concurrently the probable errors of differences are being reduced. If results in the same sense as the present ones continue to appear (as seems to be the case) they are bound presently to become very convincing to such persons as are not prevented by prejudice from accepting or appreciating scientific evidence on the problem of the effect of parental alcoholism upon the progeny.

We may evaluate our results in general terms as follows:

1. There is no evidence that specific germinal changes have been induced by the alcoholic treatment, at least in those germ cells which produced zygotes.

2. There is no evidence that the germ cells which produced zygotes have in any respect been injured or adversely affected.

3. The results with poultry are in apparent contradiction to the results of Stockard, Cole and some others with mammals.[6] I believe, for reasons which will presently appear, that this contradiction is only apparent and not real, paradoxical as such a statement may seem.

4. The results with poultry are in a number of important respects in essentially complete agreement with those of Elderton and Pearson (7) on parental alcoholism in man, of Nice (16) with mice and of Ivanov (12) with rabbits, guinea pigs, dogs and sheep. Elderton and Pearson (p. 32) summarize their investigation in the following words:

> To sum up then, no marked relation has been found between the intelligence, physique or disease of the offspring and parental alcoholism in any of the categories investigated. On the whole the balance turns as often in favour of the alcoholic as of the non-alcoholic parentage.

[6] I make no mention of the results of Ceni (2) with fowls simply for the reason that his work on this question seems to me so uncritical that I am unable to consider it seriously. His method of administration of the alcohol, the very small number of animals dealt with, the absence of any quantitative data regarding the progeny, the total absence of controls, and the obviously pathological element in the stock and the experiment generally, are sufficient grounds, it seems to me, for regarding Ceni's contribution as of no significance, either one way or the other, in the discussion of this problem. If it were worth the space in this journal I could show point by point in detail wherein Ceni's paper is without real meaning. Furthermore after a careful study of this and other papers by the same author I am confident that I could repeat his alcohol experiments with poultry and get the same results. If one applies some deleterious agent plentifully, and then so manages the environmental conditions that the birds have not even a fighting chance for normal, healthy life it is easy to produce a very sorry lot of chickens. Ceni's investigation obviously started from the point of view that alcohol was going to harm his chickens. From the account given it is difficult to conceive how that happy consummation could have been avoided under the conditions.

They further find (p. 31) that:

> The general health of the children of alcoholic parents appears on the whole slightly better than the health of the children of sober parents. There are fewer delicate children and in a most marked way cases of tuberculosis and epilepsy are less frequent than among the children of sober parents. The source of this relation may be sought in two directions; the physically strongest in the community have probably the greatest capacity and taste for alcohol. Further the higher death rate of the children of alcoholic parents probably leaves the fitter to survive.

Nice (*loc. cit.*, p. 146) summarizes his studies of the effect of parental alcoholism upon the growth of the progeny in the following way: "Although the young of the alcohol mice when given alcohol themselves excelled all the other mice in growth, other young of these same mice [i.e., of alcoholic parentage] when not given alcohol grew even faster." Ivanov, in his experiments on artificial insemination, has obtained normal offspring, which lived and made normal growth, from rabbits, guinea pigs, dogs, and sheep, where the spermatozoa used to fertilize the female were actually immersed at the time of fertilization in solutions of ethyl alcohol ranging in strength from 0.5 per cent to 10 per cent.

These various results are not to be dismissed in so light and cavalier a manner, and without reasons given, as they have been in some recent reviews of the literature. The memoir by Elderton and Pearson is a masterpiece of statistical research, sane and temperate to a degree in its conclusions. Nice's study of mice seems to be a sound, thorough and careful piece of experimental work, quite the equal in respect of its technique and its logic, of any experimental work which has been done in this field.

In attempting the interpretation of these results we are confronted with several possibilities. In the first place it might be maintained that there are fundamental physiological differences between birds and mammals, of such extent and degree as to make the action of alcohol and similar substances upon the germ cells totally different in kind in the two cases. While such a possibility can not be categorically denied, it seems to me

to be highly improbable. In the first place my results agree fully with Stockard's so far as concerns the effects produced upon the treated animals themselves. This would imply that the physiology of reproduction is alone so different in kind in birds and mammals that it is differentially affected. But it seems to me that all we know about the matter agrees in indicating that the fundamentals of ovarian and testicular physiology are essentially the same in birds and mammals.

A second possibility is that while the effect of alcohol upon the germ cells is the same in kind in birds and mammals, it differs markedly in degree in the two cases, the germ cells of birds being much more resistant to injury by alcohol than those of mammals. This seems to offer a valid explanation of the apparent discrepancy in results, and I shall return later to a more detailed discussion of it.

A third possibility is that what has here been called the total germinal dosage (cf. I), is too low to produce any effect, and that with higher dosage harmful results would have manifested themselves. There are three things here to consider. One is that the average duration of treatment prior to the birth of offspring (i.e., germinal dosage) is certainly as long, and perhaps even longer than that which has been definitely shown by Stockard to be necessary to produce deleterious results in guinea pigs. While Stockard has not, so far as I am able to find, anywhere given definite figures for total germinal dosage in connection with particular individual matings, it is clear from the general context of all his papers that a few months continuous treatment of the parents with alcohol prior to conception is amply sufficient to injure the germ cells to the point where defective offspring are produced. In some cases he apparently has got results with very short duration of treatment. Thus he says (35, p. 656): "A number of experiments in which the treatment of a female was commenced at the beginning of pregnancy have so far given rather indefinite results, although a slight effect may be indicated." This of course is a great reduction of germinal dosage and no one could expect marked or definite results. Stockard points out in many places in his

papers that as the duration of the treatment is prolonged the injurious effects on the germ cells get worse. This is of course what one would expect, but the point is that he was able to show positively harmful effects on the progeny in the early stages of his experiments when the treatment prior to conception had not been greatly prolonged, and the results of these matings early in the course of his experiments are, quite properly of course, included in the latest summaries of the investigations (cf. Stockard and Papanicolaou 38). Furthermore Stockard (cf. 34, p. 381) as well as Fèrè earlier, showed that the hen's egg is very easily influenced by alcohol during incubation and caused to develop teratogenetically.

The second point is that a careful study of the present results makes it impossible to assert that the treatment of the parents has had no effect upon the progeny, which would be logically necessary if one holds simply that the dosage has been too low to be effective. The offspring of the alcoholists, as a class, are indubitably differentiated from the offspring of the non-alcoholists. The probability that the former group is a random sample from the latter, when all 12 of the characters dealt with are taken into account, is so small as to amount to practical impossibility. The treated matings, by and large, plainly give better results in a number of respects than the controls. With all the critical precautions which were taken with the experiments this can only mean that the treatment has produced an effect. Altogether it seems impossible to explain the results of these experiments on the supposition that the duration of treatment prior to conception was not long enough to produce any effect whatever.[7] It is quite clear that the validity of the present experiments can not be challenged on the

[7] In this connection it is very difficult to refrain from discussing the 1916 results which are coming to hand as I write. Since in adequately reporting the results of an experiment of this sort it is essential to present the original data in detail, limits of space demand that a data limit be set on progress reports. As set forth earlier, the present paper reports the results up to February 1, 1916, only. It must suffice here merely to say that after 18 months continuous daily alcoholic treatment of both parents we are still getting results in the offspring essentially like those here reported.

ground of too low germ dosages as compared with mammalian experiments until we have definite statistics regarding mammalian experiments which shall show for each individual separate mating, in actual tabulated figures (a) the total germinal dosage prior to conception, using the term 'germinal dosage' in the sense defined in this paper, (b) the number and quality of offspring.

We may now return to the further consideration of the second possibility mentioned above, namely that the apparent discrepancy between the avian and mammalian results is fundamentally due to a difference in degree of resistance of the germ cells to alcohol. On this basis it is possible, I believe, to frame an hypothesis which will bring together in a satisfactory manner under one point of view the apparently discrepant results of Stockard, Pearson and the writer.

At the outstart let us remind ourselves of a point which one is apt to overlook in considering results of this sort, namely that the germ cells which produce the zygotes, which are the progeny of our experiments, are only a very minute fraction of all the germ cells which the parents form. Let X be the total number of germ cells (ova or spermatozoa) which the individual produces, and let a be the number which succeed in taking part in the formation of zygotes, and let A be the number which do not so succeed. Then, of course, $A = X - a$, or put the other way about,

$$X = A + a$$

This is the fundamental gametic equation. Starting from this point let us attempt to develop, very briefly, a general theory of the action of deleterious agents upon germ cells, and then compare our experimental results with such a general theory. We know that A is enormously greater than a. There is furthermore a great deal of evidence that a is not a random sample of X, but on the contrary is a highly selected sample. To Roux in his 'Kampf der Theile' is to be given the credit for first pointing out what now seems axiomatic, that there is constantly going on a struggle for survival among the cells of the organism, the physiologically 'best' being the survivors. To the

philosophical breeder of animals nothing seems more certainly established than that this process of selection is constantly going on and is of very special importance among the germ cells. Direct and convincing observational and experimental proof of it has been given for ova by von Hansemann (9). The double mating experiments which Cole and Davis and Cole and Bachhuber have carried out, prove the same point.

We may represent the general facts about variation in the vitality or vigor or survival value of germ cells with considerable probability by a diagram like figure 4. This diagram represents the whole population X of germ cells (ova and spermatozoa). On the base the degrees of physiological vigor or survival value are laid off in 10 equal intervals. 1 denotes a weak, poor sperm (or ovum) unlikely to survive or take part in the formation of a zygote. 9 denotes a strong highly vigorous sperm, practically sure to survive and enter upon zygote formation, except under the most adverse circumstances. The curve, which is the normal or Gaussian curve of errors, denotes the probable frequency of the different degrees of physiological vigor among the germ cells.

Now to return for a moment to our fundamental gametic equation,

$$X = A + a,$$

it is obvious that both of the groups A and a may include germ cells of varying degrees by physiological vigor, a fact which we may express symbolically in this way:

$$A = A_1 + A_2 + A_3 \ldots\ldots$$
$$a = a_1 + a_2 + a_3 \ldots\ldots$$ [8]

[8] The degree of variation within the subgroups A and a will be given, as usual, by the standard deviation of those subgroups, and these standard deviations we may indicate in the usual way as σ_A and σ_a. The quantity

$$S = \frac{\sigma_a}{\sigma_A},$$

is theoretically a very important one. It measures the relation between the variabilities of the parts of the total germ cell population which do and which do not produce zygotes. Ordinarily we may suppose that S will be less than 1, but there is no *a priori* reason why it should not be $\leqq 1$.

Suppose some deleterious agent, such as alcohol, to act upon the germ cell population X with an intensity P, the value of P being less than that required to kill all the germ cells at once. It is a reasonable assumption in accordance with known physiological laws that effect produced by the agent will be proportioned to the initial distribution of physiological vigor among the cells. The weakest germ cells will be killed, those a little stronger will be severely injured and so on. It is legitimate to assume that for values of the intensity factor P which are below a certain level, say P_m there will be a certain proportion of germ cells, which because of their high innate physiological vigor are very resistant, will not be sensibly affected by the harmful agent at all. The effect of the agent upon our fundamental gametic equation will be to introduce a new term. Using

Fig. 4 For explanation see text.

primes throughout to indicate the conditions after the deleterious agent has acted we shall have this result—

$$X' = A' + (a' + b') \qquad \text{(ii)}$$

where A' denotes the number of germ cells which do not take part in forming zygotes, $(a' + b')$ denotes those germ cells which do enter into zygote formation, a' being those physiologically vigorous cells which are highly resistant, and form vigorous and perfect zygotes, and b' denoting the less vigorous germ cells which are injured, but not put entirely out of commission, by the deleterious agent, and because of their injuries produce defective offspring.

The relations between these several parts of the germ cell population before and after the action of a harmful agent are of interest. It would seem probable that the following relations must hold finally.

$A' > A$, because a loses to A' as result of the agent's action
$(a' + b') < a$, for the same reason.

The relation of a' to b' will vary for different species, and even probably individuals, depending upon the relative resistance of the cells at intensity P. As P for any really effectively harmful agent increases, a' will become smaller, approaching 0 as a limit, when no normal offspring will be produced. This brings us to an interesting paradox, viz., that so long as $a' > 0$, that is so long as any normal offspring are produced at all, the quality of these normal offspring will be higher the greater the value of P, that is the more intense the action of the deleterious agent. This is obvious on biological grounds, because the more intense the action of the harmful agent, the more intense the selection: hence only the very 'best' germ cells will survive and make zygotes.[9]

For any given organism, deleterious agent, and intensity of dosage or application, there should be a stable relation between a', b', A' and P.

Let us now return to our experimental data in the light of these theoretical considerations. The essential point to Stockard's results, as I understand them, is that the value of P for alcohol on guinea pigs is relatively so high that practically speaking $a' = 0$ and the fundamental gametic equation has become

$$X' = A' + b'.$$

If a' has any value it is very small. One gathers that after a sufficiently high degree of alcoholization of the guinea pigs a

[9] The symbolic proof of the point is simple. Let M_a denote the mean vigor of the a germ cells, and $M_{a'}$ the mean vigor of the a' germ cell. Then we have

$$M_{a'} > M_a$$

because

$$nM_a = \sum_{a_1}^{a_m} aZ$$

and

$$n'M_{a'} = \sum_{a_h}^{a_m} a'Z',$$

where Σ denotes summation between the indicated limits, n and n' are the number of cases, Z and Z' are frequencies, and h is any subscript greater than 1 and $\leqq m$.

great majority if not, indeed, practically all of the offspring are in some degree defective. This condition of affairs I have tried to represent very roughly diagramatically in figure 5. The cross-hatched portion of the area (A') represents the sperm (or ova) which are definitely out of commission so far as taking part in zygote formation, either because of their inherent weakness or because of the action of the alcohol, or both. The singly ruled portion (b') represents the germ cells which form zygotes but have been so weakened by the alcohol that these zygotes are in some degree defective.

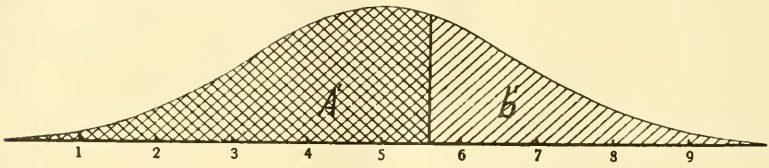

Fig. 5 For explanation see text.

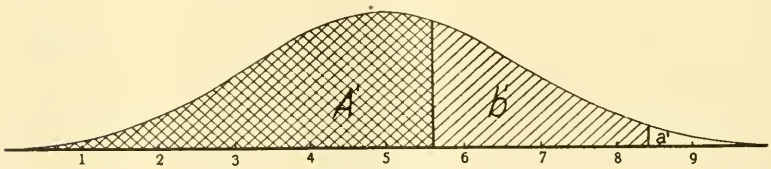

Fig. 6 For explanation see text.

A less extreme relationship between effective dosage of the agent, P, and the resisting power of the germ cells is shown in figure 6. In this diagram it will be seen that a very small part of the total area falls in the a' class, which represents germ cells of such high resisting power as not to be detectibly affected by the deleterious agent. In actual breeding experiments, involving the statistically rather small numbers which experiments with higher vertebrates must always be contented with, it would be very difficult to distinguish critically between such conditions as those represented in figures 5 and 6 respectively. It may be the case that figure 6 represents the conditions with Stockard's guinea pigs actually more truly than does figure 5. It would need larger numbers of animals and more detailed

quantitative data than he has yet published in order to reach a critical opinion. Dr. Stockard informs me, since the above was written, that in his opinion figure 6 would more fairly represent the guinea-pig case.

Now in the case of the fowls in these experiments we may suppose that the germ cells are more resistant so that the same intensity P produces an effect such as that shown in figure 7. Here relatively many more of the gametes are capable of forming normal zygotes (a'), and the b' band is much narrower, indicating that relatively few defectives are formed. The normal offspring produced are of superior quality because the alcohol has acted as a selective agency, putting completely out of commission all the poorer grades of germ cells and yet not being sufficiently intense to injure the best grades.

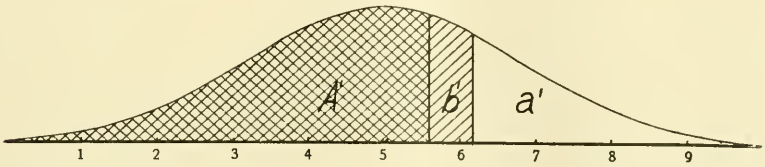

Fig. 7 For explanation see text.

In the case of the guinea pigs the selective agent, on this hypothesis, acts with such great intensity that, having put the poorer grades of germ cells out of physiological commission, it also injured all the best grades to such a degree that they produced abnormal or defective offspring.

Elderton and Pearson's results would appear to resemble the fowl results most closely. Figure 7 might be taken on this hypothesis to represent the conditions in man. Alcohol would appear to be a less intense germ cell selective agent in man than in the guinea pig. In this connection it is interesting and significant to note that Ivanov (11) found, by direct measurement of the duration of life as evidenced by movement, that the spermatozoa of the guinea pig and the rabbit *possess a relatively low degree of resistance to the action of ethyl alcohol.*

On this hypothesis it might be supposed that with larger administration to the fowls (higher germ dosage) or more years of drinking behind them in the case of Elderton and Pearson's workingmen, the conditions shown in figure 7 would gradually pass over into those shown in figure 5. Possibly this is so, but there is no evidence as yet that it is. The germ dosage index of this paper is a time index. It takes no account of the intensity factor in dosage because the intensity factor is a constant in these experiments. It is represented by the time spent in the tank in the saturated atmosphere each day. This intensity factor is the same in my experiments as in Stockard's, and in both cases it represents according to his experience and mine practically a maximum value of P. Fowls can not be left much longer than one hour at a time in an atmosphere saturated with alcohol vapor. Now in view of the facts (a) that alcohol is rapidly eliminated from the system and not accumulated therein and (b) that large and repeated doses immediately affect the germ cells very markedly as shown by Todde (40), it seems to me probable that when alcohol is administered by the inhalation method the factor which determines the width of the b' zone relative to a' is the length of time the animal stays in the tank per day, and not the number of days it is treated. It may well be that the longer the treatment is continued the greater will A' become. But all the evidence now available seems to indicate that this is at the expense of a' alone and that b' is simply pushed along or may even be narrowed.

On the hypothesis here advanced we see why the percentage of infertile eggs is higher for alcoholic than control matings. This merely is the expression of the transference of the a_1, a_2 ..... a_h germ cells over into the A' group. They are germ cells which before treatment were of rather low grade but still good enough to take part effectively in zygote formation. Alcohol treatment put them definitely over the line into the A' class. In this connection it is of interest to note that we have for the correlation between per cent of infertile eggs and germ dosage index

$$r = + 0.316 \pm 0.136$$

This result means that as the duration of treatment in days before hatching increases the proportion of germ cells falling in the A' class increases. Comparing with controls as a base this further means that this change is at the expense of the cells in the a class.

The percentage of fertile eggs hatched, the mortality of the offspring, the weight at hatching and the growth to adult weight are all superior in the progeny of alcoholists. These facts argue very strongly in favor of the present hypothesis in general, and in particular that part of it which postulates a group of germ cells a' which are of such high physiological vigor as to be effectively beyond the range of the selective agent acting at intensity P. If in respect to one or two only of these characters were the alcoholists' offspring superior we might attribute the result to accident. But when the whole series shows the same thing such an explanation is out of court. No sensible person would argue that the alcohol benefited the germ cells over so long a period of time. A selective action of the sort here postulated seems the only reasonable explanation of the objective experimental facts. It is known that an immediate, but transitory, stimulating effect may follow the administration of very minute doses of substances which in higher dosages act as poisons. This was shown to be the case by Braconnot, working about the middle of the last century on the effect of various substances upon the sensitive plant Mimosa. Czapek (5, p. 883) in commenting upon Braconnot's result especially mentions that, in spite of its potentially great importance, no later investigator has systematically followed it up. Prof. C. M. Child informs me that he has obtained exactly the same kind of a result in his studies on the effect of such substances as KCN and alcohol upon planarians. In the case of the present experiments, however, it could hardly be maintained that this primary stimulating effect of a dilute poison would continue regularly and constantly to appear after the poison had acted on the same individuals continuously for months! For such a supposition there appears to be no warrant in any known biological facts.

XII. SUMMARY

This paper deals with the effects produced upon the progeny of fowls after treatment of the parents with either (a) ethyl alcohol, or (b) methyl alcohol, or (c) ether. The chief results may be summarily stated as follows:

1. The proportion of fertile eggs, i.e., eggs in which a zygote was formed by the union of sperm and ovum, was materially reduced in the matings in which one or both individuals had been treated. The higher the germ dosage index for the mating the smaller was the percentage of fertile eggs found to be.

2. The prenatal mortality, measured by the precentage of embryos (zygotes) which died before hatching to all embryos formed, was materially smaller in the case of offspring from matings in which one or both parent individuals were treated, than in the case of offspring from untreated control parents.

3. The post natal mortality at all ages was materially lower in the case of offspring from matings in which one or both individuals were treated, than the average mortality of individuals from untreated control parents. The only matings of untreated individuals showing as low a rate of mortality as the treated matings were a selected group picked as having the very lowest mortality.

4. The sex ratio of the progeny was not sensibly affected by the treatment of the parents.

5. There was no significant difference in mean hatching weight between the offspring of treated males and the offspring of normal untreated control males when both were mated to normal untreated females. The slight differences which did appear were of the same order of magnitude as their probable errors.

6. Both the male and female offspring of matings in which both parents were treated showed a higher mean hatching weight (i.e., are heavier when hatched) than the offspring of either completely normal control matings, or of matings in which the father only was treated.

7. The offspring of alcoholized parents, whatever the nature of the mating, showed a higher mean adult body weight than

offspring of untreated parents of the same breeds mated in the same way. This is true of both sexes.

8. In the case of the male chickens there was no substantial difference in the rate of growth in the three lots until after an age of about 100 days was passed. From that point on the male offspring of treated ♂♂ × untreated and treated ♀♀ grew at a more rapid rate than the controls. The differences in mean body weight for a given age became increasingly large as the age advanced.

9. In the case of the female chickens there was no substantial difference in the rate of growth in the three lots until after an age of about 150 days was passed. During the next 25 days the controls grew faster than the chicks from treated parents. At and after 200 days of age, however, the offspring of treated parents (one and both) showed a higher mean body weight than the controls.

10. At all ages in the case of the male chicks, and in all ages but two (12.5 and 19.5 days) in the case of the female chicks, the mean body weight of the offspring having both parents alcoholic was higher than that of the offspring having one parent only, the father, alcoholic.

11. The proportion of abnormal chicks produced from treated parents was no greater than that produced from untreated parents.

12. The normal Mendelian inheritance was in no way affected by the treatment of the parents, so far as concerns any of the numerous characters observed and tested. This statement applies only to phenomena of dominance, recessiveness and sex linkage. Other Mendelian phenomena have not as yet been tested in these experiments.

13. There was no evidence from these experiments that the treatment of individual fowls, whether male or female, with either ethyl alcohol, methyl alcohol, or ether, had any deleterious effect upon those germ cells which formed zygotes. The treatment rendered many germ cells incapable of forming zygotes at all, but those which did form zygotes had plainly not been injured in any way.

14. There was no evidence that specific germinal changes have been induced by the treatment, at least so far as concerns those germ cells which produced zygotes.

15. It is suggested that these results, as well as the results of earlier workers, may be most satisfactorily accounted for on the hypothesis that alcohol and similar substance act as selective agents upon the germ cells of treated animals. The essential points in such an hypothesis may be put in the following way.

a. Assume that the relative vigor, or resisting power of germ cells varies or grades continuously from a low degree to a high degree and further assume that the absolute vigor of the whole population of germ cells, measured by the mean let us say, is different for different species.

b. In the intensity of dosage employed in inhalation experiments alcohol does not destroy or functionally inactivate all germ cells. The proportionate number of the whole population of germ cells which will be inactivated by such dosage may fairly be supposed to depend upon the mean absolute vigor or resisting power characteristic of the particular species or strain used. In a species with germ cells of absolutely low mean vigor proportionately more will be functionally inactivated than in a species of high absolute mean vigor of germ cells.

c. Besides the germ cells which are wholly inactivated by the deleterious agent, and which we may designate as class (*a*), we may fairly assume that there is a possibility of two other classes existing, viz., (*b*) germ cells which, while not completely inactivated, are so injured by the agent as to produce zygotes which are measurably defective in some degree, and (*c*) germ cells which are not measurably affected by the agent at all in the dosage employed, and produce zygotes which are not discernibly otherwise than perfectly normal.

d. It appears entirely fair to assume that germ cells of the (*a*) class are of relatively the lowest mean vigor or resisting power, class (*b*) next, and class (*c*) the highest. The proportionate number of the two sorts of zygotes corresponding to classes (*b*) and (*c*) of germ cells which would be expected to appear in

any experiments made to test the point would clearly be a function of the mutual relationship or proportionality between two variables, the dosage of the deleterious agent on the one hand, and the mean absolute resisting power of the germ cells characteristic of the strain or species of animal used in the experiments on the other hand.

e. If the dosage of the agent be relatively high in proportion to the mean absolute resisting power it would be expected that all the germ cells would fall into classes (*a*) and (*b*), producing no zygotes at all or zygotes in some degree defective. This about represents the condition, so far as can be judged from the data given, with Stockard's alcoholized guinea pigs and Weller's lead-poisoned guinea pigs. The dosage is sufficiently high in proportion to the absolute germinal resisting power that all or practically all of the offspring are defective in greater or less degree and in reference to some one or more characters. Stockard's F_2 and F_3 results indicate that though the untreated F_1 animals from alcoholists may appear normal, they really are somewhat defective.

f. If, on the other hand, the dosage, though absolutely the same, be relatively lower in proportion to the mean absolute resisting power of the germ cells it would be expected that all three germ cell classes (*a*), (*b*) and (*c*) would be represented. The zygotes actually formed would be chiefly produced by (*c*) germ cells, and to a much smaller extent by (*b*) cells. Under these circumstances it would necessarily follow that a random sample of the zygotes produced after the action of the deleterious agent would, on the average, be superior in respect to such qualities as growth, etc., which may be supposed to depend in part at least upon germinal vigor, to a random sample of zygotes formed before the action of the agent, because the germ cells of class (*c*) are a selected superior portion of the total gametic population.

g. Essentially that proportionality between effective dosage of the deleterious agent and absolute resisting power of the germ cells outlined in the preceding paragraph (f) is believed to have obtained in the present experiments with fowls, Nice's experi-

ments with mice, and nature's experiments with the working-men's population studied statistically by Elderton and Pearson.

The experiments here reported are being continued.

XIII. APPENDIX

In this appendix are given the frequency distributions of the growth measurements, on which the constants of tables 11, 12, and 13 in the text are based.

TABLE I

Untreated ♂♂ × untreated ♀♀. Ages: 2 days and 5.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	AGE = 2 DAYS		AGE = 5.5 DAYS	
	♂	♀	♂	♀
26.5				1
28.5	2	3		0
30.5	2	4	2	0
32.5	4	8	1	5
34.5	3	7	0	0
36.5	1		1	1
38.5			1	3
40.5			5	1
42.5			1	7
44.5			4	5
46.5			2	1
48.5			1	4
50.5			2	
Totals.....	12	22	20	28

TABLE II

Untreated ♂♂ × untreated ♀♀. Ages: 9 days, 12.5 days, 16 days, 19.5 days, 23 days and 26.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	9 DAYS		12.5 DAYS		16 DAYS		19.5 DAYS		23 DAYS		26.5 DAYS	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
37	1		1	1								
42	1			2								
47	2	5		1	1							
52	6	5	1	1	1							
57	3	9	5	5	1	2						
62	1	1	3	3	2	3		1				
67		1	2	6	3	4		3				
72		1	4	7	2	4	2	1	1			
77			4	2	3	8	1	5	0			
82					1	0	1	2	0	1		1
87						0	1	0	1	2		0
92						1	2	2	1	1		0
97							4	5	3	0		2
102							0	2	1	4		1
107							3	2	0	4	1	4
112							2	1	1	4	0	0
117							1	3	3	4	1	3
122							2	1	2	1	1	1
127									0	0	5	1
132									0	0	1	1
137									0	0	2	7
142									0	0	0	0
147									0	0	4	2
152									0	0	0	0
157									1	1	3	3
162											0	1
167											1	1
Totals....	14	22	20	28	14	22	19	28	14	22	19	28

TABLE III

Untreated ♂♂ × untreated ♀♀. Ages: 37 days and 40.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	37 DAYS		40.5 DAYS	
	♂	♀	♂	♀
134.5			1	1
144.5				
154.5				
164.5			1	1
174.5		1		3
184.5	1		1	2
194.5		1	1	
204.5	1	1	1	2
214.5			2	2
224.5	1	3	1	1
234.5	2	6		2
244.5	2	4	1	5
254.5	1	3	2	1
264.5	2	1	2	1
274.5	1	1	5	2
284.5			1	2
294.5				
304.5		1		
Totals.....	11	22	19	25

TABLE IV

Untreated ♂♂ × untreated ♀♀. Age: 53 days

WEIGHT IN GRAMS (CENTER OF CLASS)	53 DAYS	
	♂	♀
229.5	1	
249.5		
269.5		1
289.5	1	1
309.5		1
329.5	2	4
349.5	1	7
369.5	1	5
389.5	8	7
409.5	2	10
429.5	2	3
449.5	2	2
469.5	4	3
489.5		1
509.5	2	
529.5	3	
Totals.....	29	45

TABLE V

Untreated ♂♂ × untreated ♀♀. Ages: 67 days, 77.5 days, 95 days, 119.5 days, 147.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	67 DAYS		77.5 DAYS		95 DAYS		119.5 DAYS		147.5 DAYS	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
374.5	1									
424.5	1	2								
474.5		3								
524.5	4	9								
574.5	4	12		1						
624.5	7	10		2	1					
674.5	6	4	1	6		1				
724.5	5	4	3	3						
774.5	5		2	7		1				
824.5	1		1	2		6		1		
874.5			2	3	1	8				
924.5			3		1	13	2			
974.5			2		7	5		1		
1024.5					4	6		8		
1074.5					4	3	2	3		
1124.5					2	1	1	6		
1174.5					3		3	5		
1224.5					3			4		
1274.5					2		3	4		2
1324.5					1		1	11	1	2
1374.5							4	2		4
1424.5							3	4	1	8
1474.5					1		8			8
1524.5							2	1		6
1574.5								1		7
1624.5									4	5
1674.5									1	3
1724.5									1	2
1774.5							3		4	1
1824.5							3		3	1
1874.5									6	
1924.5									6	
1974.5									1	2
2024.5									2	
2074.5										
2124.5									2	
2174.5									1	
2224.5									1	
2274.5									1	
2324.5									1	
Totals.....	34	44	14	24	30	44	35	51	36	51

TABLE VI

Untreated ♂♂ × untreated ♀♀. Ages: 165 days, 174.5 days, 209.5 days, 267 days, 286 days

WEIGHT IN GRAMS (CENTER OF CLASS)	165 DAYS		174.5 DAYS	209.5 DAYS	267 DAYS	286 DAYS
	♂	♀	♀	♂	♀	♀
1424.5		2				
1474.5		3				
1524.5		2				
1574.5		4	2			1
1624.5		6	2		1	1
1674.5	1	7			1	1
1724.5		8	6		2	
1774.5		3			2	
1824.5						
1874.5	1	2	2			3
1924.5	2	3	3		3	3
1974.5		3	2			4
2024.5	1	3	2		2	1
2074.5	3	1	2		3	4
2124.5	5		1	4		1
2174.5	1	2	1	1		
2224.5	2	1	2	2	1	3
2274.5	1					1
2324.5	2			2		
2374.5				1		
2424.5	1			9	1	
2474.5				2		2
2524.5	1					
2574.5				3		
2624.5				4		
2674.5				2		
2724.5				4		
2774.5						
2824.5				1		
Totals.....	21	50	25	35	16	25

TABLE VII

Treated ♂ × untreated ♀♀ and treated ♂ × treated ♀♀. Age: 5.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	TREATED ♂ × UNTREATED ♀♀		TREATED ♂ × TREATED ♀♀	
	♂	♀	♂	♀
26.5		1		
28.5	1	0		
30.5	2	2		1
32.5	2	1	1	2
34.5	6	1	0	1
36.5	7	7	2	0
38.5	9	5	1	2
40.5	6	12	0	3
42.5	10	13	4	7
44.5	7	5	10	9
46.5	5	4	3	4
48.5	6	3	3	3
50.5	2	4	2	1
52.5	1	0	3	
54.5		0		
56.5		1		
Totals.....	64	59	29	33

TABLE VIII

Treated ♂ × untreated ♀♀ and treated ♂ × treated ♀♀. Age: 12.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	TREATED ♂ × UNTREATED ♀♀		TREATED ♂ × TREATED ♀♀	
	♂	♀	♂	♀
35.5		2		
39.5	1	0		
43.5	9	4	3	1
47.5	6	8	1	7
51.5	7	10	5	5
55.5	10	6	5	9
59.5	5	7	7	5
63.5	9	8	1	3
67.5	3	9	5	0
71.5	2	1	1	1
75.5	2		1	0
Totals.....	54	55	29	31

TABLE IX

Treated ♂ × untreated ♀♀ and treated ♂ × treated ♀♀. Age: 19.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	TREATED ♂ × UNTREATED ♀♀		TREATED ♂ × TREATED ♀♀	
	♂	♀	♂	♀
42		2		
47	3	0		
52	0	0		
57	0	1	1	1
62	3	2	1	1
67	2	6	0	5
72	6	9	3	4
77	6	5	2	2
82	6	6	5	4
87	5	3	3	3
92	4	6	3	1
97	7	6	4	3
102	3	4	2	3
107	4	4	3	1
112	2	1	1	0
117		1	0	1
122			0	
127			0	
132			1	
Totals.....	51	56	29	29

TABLE X

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Age: 33.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	TREATED ♂♂ × UNTREATED ♀♀		TREATED ♂♂ × TREATED ♀♀	
	♂	♀	♂	♀
94.5			1	
104.5		2		
114.5	2	2		
124.5	1	3	1	2
134.5	2	2		2
144.5	4	7		
154.5	2	4	2	3
164.5	10	5	4	3
174.5	6	4	2	4
184.5	4	4	1	4
194.5	4	7	5	7
204.5	5	5	3	
214.5	4	3	3	1
224.5	3	3	1	1
234.5	2	2	2	1
244.5			1	
254.5	1			
264.5		1		
274.5	1			
354.5			1	
Totals.....	51	54	27	28

TABLE XI

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Ages: 47.5 days and 68.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	47.5 DAYS				68.5 DAYS			
	Treated ♂ × untreated ♀		Treated ♂ × treated ♀		Treated ♂ × untreated ♀		Treated ♂ × treated ♀	
	♂	♀	♂	♀	♂	♀	♂	♀
209.5	1	3						
229.5	1	2	1					
249.5	3	11		1				
269.5	6	3	1	5				
289.5	3	4	3	4				
309.5	9	7	1	5				
329.5	7	6	4	3				
349.5	2	3	4	6				
369.5	6	7	4	1				
389.5	5	3	3	1			1	
409.5	3	1	3			3		1
429.5			3	1	1	2		
449.5	1					3		1
469.5		2	1		3	4		
489.5						2		
509.5					2	4	1	2
529.5						3	1	2
549.5					4	4		5
569.5					4	2	2	3
589.5	1				2	1	1	2
609.5					7	5	4	3
629.5					5	2	1	2
649.5					3	1	3	4
669.5					4	6	2	2
689.5					2	3	2	1
709.5					2			
729.5					2		2	
749.5					4		4	1
769.5						2		
789.5					1		1	
809.5							2	
829.5							1	
Totals..	48	52	28	27	46	47	28	29

TABLE XII

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Ages: 89.5 days, 110.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	89.5 DAYS				110.5 DAYS			
	Treated × ♂ untreated ♀		Treated ♂ × treated ♀		Treated ♂ × untreated ♀		Treated ♂ × treated ♀	
	♂	♀	♂	♀	♂	♀	♂	♀
424.5		1						
474.5								
524.5								
574.5								
624.5	1	2	1	2				
674.5		3				1		
724.5	1	4		1				
774.5	3	8	2	3				
824.5	1	7	1	6		1	1	
874.5	6	7	1	8		1		1
924.5	9	9	4	3		3		
974.5	5	2	2	4		2		1
1024.5	9	7	3	2	2	10		4
1074.5	7	1	4	1	1	4	1	4
1124.5	5	1	1		1	5		7
1174.5	3		4		2	7	1	3
1224.5	1				5	6		5
1274.5			1		3	7	3	1
1324.5			1		2	2	3	2
1374.5			1		9	3	2	2
1424.5					5		5	
1474.5					8		2	
1524.5					5		3	
1574.5					1		2	
1624.5					2		2	
1674.5					5			
1724.5							1	
1774.5								
1824.5								
1874.5							1	
Totals..	51	52	26	30	51	52	27	30

TABLE XIII

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Ages: 138.5 days, 166.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	138.5 DAYS				166.5 DAYS			
	Treated ♂ × untreated ♀		Treated ♂ × treated ♀		Treated ♂ × untreated ♀		Treated ♂ × treated ♀	
	♂	♀	♂	♀	♂	♀	♂	♀
1049.5		1						
1149.5		2		1				
1249.5		3	2	1		1		
1349.5		11		7		1		1
1449.5	2	11		12		5		1
1549.5	2	19	1	3		12		5
1649.5	5	3	1	3		7	1	12
1749.5	10	2	7	3	1	13		3
1849.5	11		4		3	5		3
1949.5	6		4		4	5	2	3
2049.5	7		5		7	2	2	2
2149.5	5		2		3		6	
2249.5	2		1		9		4	
2349.5					6		2	
2449.5			1		8		5	
2549.5					2		3	
2649.5					5		2	
2749.5					1		1	
2849.5								
2949.5							1	
Totals..	50	52	28	30	49	51	29	30

TABLE XIV

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Ages: 194.5 days 204.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	194.5 DAYS				204.5 DAYS			
	Treated ♂ × untreated ♀		Treated ♂ × treated ♀		Treated ♂ × untreated ♀		Treated ♂ × treated ♀	
	♂	♀	♂	♀	♂	♀	♂	♀
1649.5		2		2				
1749.5		1		3				1
1849.5		2		2		1		1
1949.5		2		2		2		2
2049.5		4						2
2149.5		2		3		2		1
2249.5	3	1		1				
2349.5	3							
2449.5	2		2	2	1			1
2549.5	2				2		1	
2649.5	4		4					
2749.5	1		2					
2849.5	2		2		1		2	
2949.5	1		1		1		1	
3049.5	1		1					
3149.5								
3249.5					1		1	
Totals..	19	14	12	15	6	5	5	8

TABLE XV

Treated ♂♂ × untreated ♀♀ and treated ♂♂ × treated ♀♀. Age: 290.5 days

WEIGHT IN GRAMS (CENTER OF CLASS)	TREATED ♂ × UNTREATED ♀	TREATED ♂ × TREATED ♀
	♀	♀
1474.5		1
1524.5 to 1774.5 inclusive		
1824.5	1	1
1874.5		
1924.5	1	
1974.5	2	1
2024.5	2	
2074.5		1
2124.5	2	2
2174.5		2
2224.5		
2274.5	1	2
2324.5		
2374.5		
2424.5		
2474.5		
2524.5		
2574.5		1
Totals.....	9	11

EFFECTS OF CENTRIFUGAL FORCE ON THE STRUCTURE AND DEVELOPMENT OF THE EGGS OF CREPIDULA

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ONE HUNDRED AND TWENTY-FOUR FIGURES

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1. INTRODUCTION

1. Historical

The use of very strong centrifugal force in the study of the structure and development of the egg was first made, I believe, by Gurwitsch ('04) and Lyon ('06). Before that time the effects of a rather weak centrifugal force of from 4 to 20 times gravity on the development of eggs had been studied by Rauber ('84), Roux ('84), O. Hertwig ('99, '04), Morgan ('02), and Wetzel ('04); but Gurwitsch used a very strong centrifugal force by which he injured or destroyed the protoplasm for the purpose of analyzing its structure, while Lyon used a force of from 4500 to 6400 times gravity in order to study its effects on development. Lyon discovered that by means of this great force the substances in the eggs of Arbacea, Asterias, Chaetopterus, Phascolosoma, and Cynthia could be separated into three or four layers differing in color or refractive index, and he made a brief study of the development of the centrifuged eggs of Arbacea.

This work was quickly followed by extensive studies of the development of centrifuged eggs of Chaetopterus by Lillie ('06, '09) and of the centrifuged eggs of Arbacea, Cumingia, Cerebratulus, Hydatina, the fish and the frog by Morgan ('07, '09, '10). Boveri ('10) and Hogue ('10) studied the effects of strong centrifugal force on the eggs of Ascaris; Conklin ('10) on the eggs of Physa, Lymnaea and Planorbis; Konopacki ('11) on the eggs of the frog, and Jenkinson ('14) also on the eggs of the frog.

In general it has been found that yolk, which is usually the heaviest substance in the egg, is thrown to the centrifugal pole, oil or fatty substance to the centripetal pole, while the transparent cytoplasm together with the nucleus occupies the middle zone between the other two. Usually eggs develop normally after this stratification, although the distribution of oil, yolk, and pigment may be very abnormal; and even the cytoplasm may be more abundant in certain cleavage cells than in normal development, or less abundant in others, without permanently interfering with typical development.

Furthermore a general result of previous work has been to show that the polarity and pattern of organization of an egg are not changed by this dislocation of egg substances; the polarity and pattern of the embryo which develops from such an egg remains unchanged irrespective of the location of these different materials within the egg. This is a surprising fact which invites further study. How is it possible to dislocate in any axis the visible material substances of an egg and yet leave its polarity and pattern of organization undisturbed?

Lillie ('06) concludes that polarity is a property of the 'ground substance' of the egg, this substance being "a fluid which has no filar, reticular or alveolar structure," but yet is 'firmly organized' so that it is not affected by centrifuging. He regards the substances which are dislocated by centrifugal force as mere 'inclusions' in this 'ground substance,' consequently polarity remains unchanged when these inclusions are forced to occupy new positions since polarity inheres in the 'ground substance' which is not moved by centrifugal force. It is evident from Lillie's use of this term that he means the 'ground substance' to include what is commonly called cytoplasm as contrasted with metaplasm or inclusions. However it will be shown in this paper that most of the cytoplasm of an egg can be displaced without permanently changing the polarity of the egg.

In eggs which contain relatively little yolk, such as those of echinoderms, Chaetopterus, Cumingia, etc., the yolk may be thrown to any pole without greatly displacing the protoplasm from its normal position, and consequently it is possible in these cases that normal development results because the real formative materials, viz., nucleus and cytoplasm, have not been displaced to any great extent by centrifugal force. But when the volume of yolk is large, as in the egg of Crepidula, the nucleus and cytoplasm may be displaced from their normal positions by nearly the whole diameter of the egg, and the subsequent development of such eggs throws light not only upon the specific value of different egg substances but also upon the polarity and organization of the egg as a whole.

2. General aims and results of this work

It was with a view to determine more exactly whether there is a 'ground substance' which remains unmoved in centrifuged eggs, or whether the morphogenetic substances of the egg are moved with the other substances and later resume their normal positions that the following work was undertaken. The eggs upon which these experiments were performed were those of the marine gasteropod, Crepidula plana. This object was chosen not only because of my familiarity with its normal development but also because the yolk in this egg is so abundant that any change in its position involves marked changes in the positions of nuclei and cytoplasm, which are presumably parts of the 'ground substance.'

If the eggs of this gasteropod are subjected, after fertilization and before the first cleavage, to centrifugal force of approximately 600 times gravity, the yolk is thrown to the centrifugal pole, where it occupies about three-quarters of the volume of the whole egg; the middle zone, consisting of nucleus and clear cytoplasm, comprises a little less than one-quarter, and the oil zone constitutes about one sixty-fourth of the volume of the entire egg, the relative volumes of the three zones being 49: 14: 1. In normal eggs of this stage the nucleus, centrosphere and most of the cytoplasm lie near the animal pole, but in centrifuged eggs these formative substances may be displaced far from this position, the yolk, for example, being thrown to the animal pole and the protoplasm to the vegetal one, or these displacements may take place in any other axis. Nevertheless such eggs frequently develop normally, showing that the polarity and pattern of organization of the egg have not been permanently changed by this dislocation of the formative materials. It seems necessary to conclude that there is some material substance, or relation of parts, in these eggs which persists with relatively little change, in spite of the dislocations caused by centrifuging, but if there is a 'ground substance' here which is not moved by centrifugal force it must be a relatively small part of the general protoplasm of the egg.

There is good evidence, which will be presented in the descriptive part of this paper, that this is indeed the case and that, while the greater part of the cytoplasm is free to move under the influence of centrifugal force, there is in these eggs a denser, more viscid portion of the protoplasm which forms a framework running through the cell and connecting the nucleus and centrosome, or centrosphere, with a peripheral layer which surrounds the entire egg. This framework may be stretched or distorted and yet may be able to bring back dislocated parts to their normal positions unless partition walls have been formed in the meantime which prevent this return. This framework is the seat of the polarity and pattern of organization of the cell; it holds the cell organs, especially the centrosphere and the nucleus, in a definite relation to one another and to the cell axis, and it prevents the complete stratification of cell substances into sharply marked zones according to their specific weights. The substance of this framework is probably identical with the 'ground substance' of Lillie, though in Crepidula it constitutes a relatively small part of the cell contents, and in my opinion it does have a "filar, reticular or alveolar structure." Furthermore this substance is affected by centrifuging; it is stretched and distorted if centrifuging is strong enough, but is capable of recovering its normal form afterward.

It seems evident that the term 'ground substance' is not an appropriate one for this denser protoplasm, which constitutes the achromatic substance of the mitotic figures and of the resting nucleus as well as the astral radiations and strands which connect these with the peripheral layer of the cell; this denser protoplasm is much less abundant than the more fluid protoplasm which forms the chief part of the middle zone of centrifuged eggs; it is not uniformly distributed throughout the cell, but exists in astral radiations and fine strands which run through the more fluid protoplasm as well as through the yolk. It is probable, however, that it is identical with the 'spongioplasm' of Leydig, the 'kinoplasm' of Strasburger and in many respects it corresponds to the 'interalveolar substance' of Bütschli, and to the 'archoplasm' of Boveri.

This introductory account of the aims and chief results of this work will serve perhaps to make more easily intelligible the following detailed account of these experiments.

3. Material and methods

The experiments here described were begun ten years ago at the Marine Biological Laboratory at Woods Hole, Massachusetts, and have been continued there almost every summer since. More than one hundred and forty different experiments were performed and in every instance the results of these experiments were studied by means of carefully stained and permanently mounted preparations. In the earlier years of this work the eggs in their capsules were centrifuged in a machine driven by hand at such a speed as to make the centrifugal pressure about 2000 times gravity; in later years a machine driven by water pressure was used, the centrifugal force being approximately 600 times gravity. There is a slight tendency for eggs to rotate during centrifuging so that the animal pole becomes centripetal and the vegetal pole centrifugal in position, as is shown by the somewhat larger number of eggs in this position than in any other, and yet the viscosity of the fluid in which the eggs are suspended or the pressure of the thin-walled capsules upon them prevents many of the eggs from rotating. Eggs in different stages of development were centrifuged for various lengths of time. They were then removed from the centrifuge and either fixed at once or left in finger bowls of fresh sea-water for varying lengths of time, as indicated in the description of figures given at the end of this paper.

In most cases the eggs were fixed, stained and mounted entire in the method described by me in previous papers ('97, '02). These permanent preparations were made soon after the experiments were performed and they are still in good condition. Many serial sections also were cut, but in general they are less instructive than whole amounts. All drawings were made with a 3 mm. homogeneous immersion lens with which the finer details of nuclei, centrosomes and cytoplasm can be seen with great distinctness.

II. RESULTS OF CENTRIFUGING DURING MATURATION AND FERTILIZATION STAGES

In Crepidula, as in most other prosobranchs, the eggs are fertilized within the oviduct of the female and are then surrounded by secretions from the nidamental gland; the outermost layer of these secretions hardens into a capsule. By opening the oviduct of females taken in the act of egg-laying or by getting capsules immediately after they have been deposited, it is possible to obtain eggs before the germinal vesicle breaks down and before the spermatozoon enters the egg.

Under normal conditions the polarity of the unfertilized egg is marked by the eccentricity of the germinal vesicle toward the animal pole. Generally the spermatozoon enters the egg near its vegetal pole, though there may be exceptions to this rule. Under normal conditions both first and second polar bodies are formed invariably at the animal pole; indeed so general is this rule that the animal pole is frequently defined as that pole of the egg at which the polar bodies are formed, and yet, as we shall see later, the polar bodies may be caused to form at any point on the surface of the egg without in any way changing the polarity of development. In Crepidula the polar bodies never change their point of attachment to the egg; as long as they are present they remain at the point where they were extruded and they therefore constitute a valuable landmark.

1. Results of centrifuging before and during the first maturation division (figs. 1–18)

From the facts just stated it is evident that before the formation of the first polar body, there is no sure way of distinguishing the original poles of a centrifuged egg of Crepidula, though the incomplete stratification of the cell constituents, and particularly the position of the sperm nucleus and of the first maturation spindle and the direction of movement of various cell constituents after centrifuging, may indicate with a certain degree of probability the location of the original poles.

Before maturation, yolk and cytoplasm intermingle in all parts of the egg and, although the cytoplasm is somewhat more abundant at the animal pole than elsewhere, these two substances are not sharply segregated. When such an egg is strongly centrifuged the yolk is driven to the centrifugal pole, while the cytoplasm is displaced toward the opposite pole, so that these substances come to be partially segregated (figs. 1 and 2), though this segregation is never so complete as it is in later stages. The germinal vesicle goes with most of the cytoplasm to the centripetal pole. The entire egg is frequently flattened in the axis of centrifuging, but the germinal vesicle is elongated in that axis, probably owing to the fact that the nucleus is not subjected to external pressure, whereas the egg is. Before the prophase of division, the chromatin is not moved within the germinal vesicle by centrifuging; after the prophase has begun, the chromatin appears to be free to move. This corresponds more or less closely with the conditions found by Kite ('13) in his micro-dissections where the nucleus during resting stages was found to be a gel which becomes more fluid during division phases. In the prophases shown in figures 1 and 2 the chromosomes are evidently heavier than other constituents of the nucleus since they collect in the centrifugal end of the germinal vesicle while the nucleolus goes to the centripetal end of the vesicle, thus showing that in this egg the nucleolus is lighter than other nuclear constituents; in other cases it is heavier, as for example in the ovarian egg of the lobster (Herrick, '95) and in the electric-motor nerve cells of torpedo (Dahlgren, '15). The axis of the spindle in figure 2 is approximately at right angles to the axis of centrifuging, but as the initial position of the spindle bears no constant relation to the axis of the egg this fact has no particular significance.

When the membrane of the germinal vesicle of a normal egg dissolves, the first maturation spindle is left in the egg usually at some distance from the surface and at the same time the spermatozoon enters the egg usually near the vegetal pole. When eggs are strongly centrifuged at this stage there is a fairly sharp separation of cytoplasm and yolk; the maturation spindle is carried along with the cytoplasm thus showing that it is not at this

stage closely attached to the surface layer of the cell; the nucleolus of the germinal vesicle is carried to the centripetal pole of the egg where it lies with the lightest substances of the cell, but the sperm nucleus may be found now in the cytoplasm, now in the yolk and again on the border between the two, thus showing that it is not moved to any great extent by centrifugal force and that yolk or cytoplasm may stream past it without much altering its position (figs. 3–18). If the sperm nucleus were free to move according to its specific weight it would always occupy some constant position either in the cytoplasm, in the yolk, or in the region between the two; and the fact that it is not found in any constant position with regard to cytoplasm or yolk shows that something prevents its free movement. It seems, probable, therefore, that after the sperm nucleus has entered the egg it becomes attached to the cell framework by astral filaments which radiate from the sperm centrosome and which are so fine that it is difficult to see them and yet so strong that, although all the movable substances of the egg may flow past, the sperm nucleus is not torn from its moorings.

After the maturation spindle of the normal egg has reached its metaphase it becomes closely attached to the egg surface at the animal pole by one of its asters and thereafter this attachment can not be broken by the strongest centrifuging to which the eggs were subjected. Figures 5 and 6 represent such eggs in which yolk has been driven to the animal pole and cytoplasm to the opposite pole, but the maturation spindles are so firmly anchored to the surface layer of the egg that they can not be pulled away, even though the spindle itself is stretched in length and the egg surface is indented, apparently by the pull of the spindle upon it. Since this attachment of the spindle to the egg surface occurred in this case before centrifuging there is no doubt that this point of attachment represents the true animal pole of the egg.

Figures 9–12 are all from the same experiment; the eggs were centrifuged in the prophase before the first maturation spindle had become firmly attached to the surface of the egg, consequently the spindles were carried into the interior. The eggs

were then allowed to stand for one and one-half hours before they were fixed, during which time the spindles advanced to the anaphase. In these four figures the spindles lie near the middle of the egg and the egg contents are not regularly stratified but the spindles and some of the cytoplasm project into the yolk in such a manner as to suggest either that the spindles were limited in their movement at the time of centrifuging or that after centrifuging they were moving back to their original positions. In either case it seems necessary to assume that the connection of the spindles with the animal pole, probably by means of protoplasmic fibers, was never lost. Finally such spindles probably come to the surface of the egg at the animal pole and form normal polar bodies since in all eggs of this experiment which were allowed to develop further the polar bodies are typical in appearance and position, lying at the center of the ectodermal pole.

Figures 13–18 represent eggs which were centrifuged in the late anaphase of the first maturation division after the spindle was closely attached to the animal pole; in figure 13 the egg was fixed immediately after centrifuging; in figures 14 to 18 they were left in normal conditions for one and one-half hours before being fixed. In every case the spindle has remained in its original position and a normal polar body has been formed, though the cytoplasm and yolk are abnormal in position. As in previous figures the sperm nuclei show little indication of having been moved by the centrifuging. In figures 17 and 18 the lane of cytoplasm leading from the animal pole to the cytoplasmic field indicates either that the egg substances were not regularly stratified by centrifuging, or that they are beginning to return to their original positions.

If the first maturation division occurs normally it is always easy thereafter to identify the original animal pole of the egg, however much the egg substances may have been moved out of their normal positions, by the location of the first polar body. This is an invaluable landmark since as long as the polar body remains attached to the egg it does not move from the position at which it was first formed. Before the formation of the first polar

body the identification of the animal pole in centrifuged eggs is more or less a matter of conjecture, dependent largely upon the position of the egg nucleus or spindle and upon the subsequent movements of the egg substances. After the first polar body has been formed normally the position of the animal pole is no longer uncertain. However, as we have seen in figures 9 to 12, it is possible to move the first polar spindle before it becomes firmly attached to the periphery of the egg, and if such eggs are kept whirling for a long time the polar body may form at other points on the egg than the original animal pole, as shown in figures 36 to 38.

2. Results of centrifuging during the second maturation division (figs. 19–58)

In the late anaphase of the first maturation division the chromosomes in the egg can not be centrifuged away from the animal pole to which they are bound probably by the interzonal fibers (figs. 13 to 18). When the second maturation spindle has reached its early metaphase this attachment is relaxed, for if eggs are centrifuged at this stage the spindle is carried away from the animal pole along with the cytoplasm and may be transported to any part of the cell (figs. 19 to 21). However, if the second maturation spindle has reached its anaphase at the time the centrifuging begins it can not be moved very much since one pole of the spindle becomes more or less closely attached to the surface of the egg by its astral fibers. In some cases such a spindle may be pulled away from the surface for a short distance without breaking this attachment (figs. 23, 24), but in other cases it remains closely attached to the surface at the animal pole (figs. 22, 25, 29). This variation is probably due to slight differences among various eggs in the age of the spindle and in the firmness of its attachment to the surface, as well as to differences in the strength of centrifuging.

If the young maturation spindle is centrifuged away from the animal pole (figs. 19 to 21) it takes a position in the cytoplasm between the yolk on one side and the oil cap on the other. It may be carried all the way through the egg from the animal to

the vegetal pole (fig. 21), but however far it may be from the animal pole it moves back to its normal position to finish its division unless it is prevented by very long or strong centrifuging; in the latter case the polar body may be cut off wherever the spindle happens to be. Thus in eggs which are centrifuged from 30 minutes to 2 hours during the maturation divisions the polar bodies may be extruded at any point on the surface.

3. Formation of giant polar bodies (figs. 23 to 27, 31 to 58)

Most polar bodies that are formed during centrifuging and all that are formed at a distance from the animal pole are larger than normal ones, though they may vary greatly in size (figs. 32 to 58). The size of a polar body or of any cleavage cell depends upon the position of the mitotic figure at the time of cell constriction, since the partition wall between daughter cells always goes through the equator of the spindle; when one pole of the spindle is pressed against the cell membrane, as in the maturation divisions, the size of the polar body depends upon the length of the spindle. The extremely small size of normal polar bodies is due to the fact that the maturation spindle continually grows shorter during the later stages of mitosis, and whenever giant polar bodies are formed it is due to the median position of the spindle in the cell or to its elongation if it is attached to one pole.

When centrifuging occurs during the anaphase of a maturation division after the peripheral pole of the spindle is closely attached to the surface of the egg at the animal pole the spindle may become much elongated, especially if yolk is driven to that pole (figs. 6, 22 to 26). And conversely, whenever there is a stretching of the spindle, it is evident that one or both of the poles are attached to the surface layer even though this attachment may not be directly visible. Consequently, the elongation of the spindle in the formation of giant polar bodies indicates that one pole of the spindle is attached to the surface at the animal pole. The fact that a polar body may be given off at the opposite pole of the spindle, i.e., at the vegetal pole of the egg, and yet the polarity of the egg remain undisturbed and

normal development result, proves that there is no essential difference in the two poles of the maturation spindle. Also the return of the egg nucleus to the animal pole after centrifuging and after the formation of a polar body at some other pole of the egg is probably due to attachments which connect the nucleus to the animal pole.

Because of the elongation of the spindle in these centrifuged eggs, the polar body which is formed may be extraordinarily large. If centrifuging occurs during the first maturation division, the first polar body is the giant one; if during the second maturation, it is the second polar body; if during both maturation divisions, both polar bodies are abnormally large. In my experiments the giant polar body is usually the second one, since many more eggs were centrifuged during the second maturation division than during the first. The cell constrictions shown in figures 23 to 25 lie opposite the equator of the second maturation spindle and indicate that giant polar bodies are about to be formed. These giant polar bodies may be formed at the animal pole or at any other point on the surface of the egg; they may contain the oil of the light zone, the cytoplasm of the middle zone or the yolk of the heavy zone or they may contain samples of all these substances, depending upon whether they are formed at the centripetal or the centrifugal pole and also upon their size (figs. 32 to 34, 43 to 55, *etc.*).

Normal first polar bodies usually divide by mitosis, second polar bodies rarely do, but I have never seen a case in which a giant polar body divides, and this in spite of the fact that the polar body may contain samples of all the egg substances and may be as large as, or even larger than, the rest of the egg. In most cases the chromosomes of the polar bodies never form a resting nucleus nor even chromosomal vesicles but remain as distinct chromosomes up to the latest stage studied (figs. 53, 54, 56). In a few instances these chromosomes form chromosomal vesicles or even a resting nucleus (figs. 49, 50, 51). But in only one instance (fig. 57) have I observed an indication of an approaching division of a giant polar body and this case is a very doubtful one.

These 'polar bodies' may be caused to form at any point on the surface of the egg, without changing in the least the position of the ectodermal pole. It may be doubted whether such cells which are formed at some distance from the ectodermal pole can properly be called 'polar bodies,' not merely because they do not mark a specific pole but also because they are larger than normal polar bodies, they frequently contain different oöplasmic substances such as oil and yolk as well as cytoplasm, and they doubtless sometimes contain that pole of the spindle and its chromosomes which would have remained in the egg if the polar bodies had been formed normally; that is these abnormal cells differ both in position and in constitution from normal polar bodies.

On the other hand they show certain resemblances to polar bodies, viz., they rarely divide and never undergo regular cleavage, and most important of all they are formed by those peculiar nuclear divisions which are known as the maturation divisions. Since the notable work of O. Hertwig ('90) and Boveri ('91) in which they pointed out the parallelism between oögenesis and spermatogenesis it has been universally recognized that the two maturation divisions are homologous in oögenesis and spermatogenesis and that consequently the cells which are formed by these divisions are comparable. The cells formed by the first maturation divisions are known as 'second oöcytes' or 'spermatocytes,' those formed by the second maturation division as 'oötids' or 'spermatids.' In normal eggs three of these oötids are very small and are known as 'polar bodies,' while the fourth is large and is called the 'egg;' it is evident from the manner of their origin that the polar bodies are rudimentary eggs, a view which was first set forth by Mark ('81). When eggs have been subjected to pressure or to centrifugal force, all of the oötids may be of approximately the same size, although only one of these cells develops; the fact that three of these cells do not always lie at the animal pole of the cell which develops indicates that the term 'polar body' in such cases is a misnomer. But at least all four cells are oötids and for the sake of simplicity of expression it seems desirable to call these oötids, which do not develop and

which are usually smaller than the one which does, "polar bodies" whether they lie at the animal pole or not.

4. Why polar bodies do not develop[1]

Normal polar bodies then are rudimentary eggs which do not develop, though they sometimes divide once or twice. Their failure to develop is usually held to be due to their small size, but even where the polar bodies are quite large, as is sometimes the case in gastropods, polyclads and nematodes, they do not develop. In one case only has the development of a polar body, or rather of two second oöcytes, been observed. Francotte ('98) discovered in the polyclad Prostheceraeus that at the first maturation division the egg divided into two nearly equal cells; each was then entered by a spermatozoon and normally fertilized and at the second maturation division each formed a small second polar body and underwent normal cleavage and developed to the gastrula stage. In a few other instances the entrance of a spermatozoon into a polar body has been reported though some of these cases are not entirely convincing and need verification. Thus Platner ('86) described the entrance of a spermatozoon into a polar body of Arion; he maintained that the polar bodies are formed before the entrance of the sperm, which would make this case similar to that of Prostheceraeus, but the evidence offered is by no means conclusive. Sobotta ('95) calls special attention to the large size of the polar bodies in the mouse and suggests that they may be capable of being fertilized, but offers no evidence in favor of this view. Kostanecki ('91) has observed a spermatozoon with its head penetrating the second polar body of Physa, a thing which he regards merely as a 'curiosity.' Lefevre ('07) found that the same reagent (HCl) which causes the eggs of Thalassema to develop parthenogenetically also caused the polar bodies to undergo several cleavages.

The most striking difference between Prostheceraeus and other animals in which giant polar bodies have been reported is to be

[1] The substance of this section was summarized in Proceedings National Academy Sciences, 1, pp. 491–496, 1915.

found in the fact that in the former fertilization does not take place until after the first maturation division is completed and then each of the daughter cells is fertilized, whereas in the latter the entrance of the spermatozoon occurs before the completion of the first maturation division, with the result that one of the daughter cells contains a spermatozoon and the other does not.

In Crepidula the spermatozoon usually enters the egg at the time when the germinal vesicle dissolves and always before the first polar body is cut off. In many mollusks, annelids and ascidians, the first maturation spindle remains in the metaphase until the spermatozoon enters the egg or until the egg is stimulated by other means (artificial parthenogenesis) to begin development. The giant polar bodies of Crepidula behave like unfertilized eggs in these regards: 1) the chromosomes do not usually unite to form a daughter nucleus but remain as if they were in the metaphase, as in Chaetopterus, Ciona, etc., though no distinct spindle is visible (figs. 38, 40 to 46). 2) They also resemble unfertilized eggs in that the whole cell stains a purple color in picro-haematoxylin showing that cytoplasm is diffused throughout the whole cell, whereas after fertilization there is a fairly sharp separation of cytoplasm and yolk, the former alone staining purple. 3) Associated with this lack of segregation of cell substances in giant polar bodies there is a lack of the movements which in the fertilized egg lead to the segregation of cytoplasm at the animal pole and of yolk at the vegetal one.

These giant polar bodies contain samples of all the oöplasmic substances; they may be larger than the oötid which does develop, but the one thing which they lack is a spermatozoon, whereas that oötid which does develop invariably contains a spermatozoon; we must conclude therefore that the giant polar bodies of Crepidula do not develop because they are not fertilized, and they are not fertilized because a spermatozoon had entered the egg before their formation, thus rendering the polar bodies as well as the egg impervious to other spermatozoa.

In this fact is to be found the explanation of the different behavior of the giant polar bodies of Prostheceraeus and of other animals, e.g., Crepidula, for it is well known that one of the first

effects of the entrance of a spermatozoon into an egg is the prevention of other spermatozoa from entering. If the spermatozoon enters the egg before the first polar body is cut off that polar body as well as other cells which are formed from the egg are rendered 'immune' to other spermatozoa.

But although the influence of the entering spermatozoon spreads so rapidly over the egg that within a few minutes at most it renders all portions of the egg surface 'immune' to other spermatozoa and thus prevents the fertilization of polar bodies which are formed after fertilization, this influence does not go so far as to cause the polar bodies to develop, even though such polar bodies may be formed several hours after the spermatozoon enters the egg. In Crepidula the second polar body is formed about three hours after the entrance of the spermatozoon, and during this time the sperm head has grown into a vesicular nucleus and the sperm aster has become quite large, but in spite of this the spermatozoon has not sufficiently affected the egg substance to cause the second polar body to develop even though that body may contain the larger part of the egg protoplasm. Only that portion of the egg develops which contains the sperm nucleus and aster.

This conclusion is similar in many respects to that reached by Ziegler ('98), who found that when eggs of the sea urchin, Echinus microtuberculatus, were constricted by cotton fibers under pressure only that portion of the egg which contained the spermatozoon segmented while the portion containing the egg nucleus never divided, though its nucleus frequently went through the division phases, but without any division resulting. In this case the portion of the egg containing the sperm might remain for some time connected with the other portion by a narrow neck, and yet the influence of the sperm in the one half did not cause the other half to develop.

These facts are of interest because of their bearing on the nature of one of the processes concerned in fertilization. In a series of important and extensive works on artificial parthenogenesis and fertilization, which he has summarized in a recent book, Loeb ('13) has shown that at least two factors are involved

in artificial parthenogenesis, (1) an external factor, such as butyric acid, which causes a cytolysis of the cortical layer of the egg followed by increased oxidation and which leads to the rapid disintegration of the egg at normal temperatures, and (2) an internal factor, such as hypertonic solutions, lack of oxygen, etc., which inhibits this disintegration. Loeb concludes also that in normal fertilization both of these factors are present and that the spermatozoon carries substances into the egg which (1) cause cytolysis of the cortical layer and increased oxidation and (2) other substances which inhibit this cytolysis before it leads to the disintegration of the egg. Godlewski ('11) also finds that the cytolysis which is caused by fertilizing the eggs of sea urchins by the sperm of Chaetopterus may be checked and artificial parthenogenesis induced by a brief treatment of such cross fertilized eggs with hypertonic sea water.

R. S. Lillie ('11) concludes that the cortical changes consist in increased permeability of the cell membrane which tends "to destroy the normal osmotic equilibrium and allow abnormal diffusion of substances into and out of cells." The essential result of the after treatment of such eggs with hypertonic sea water is to decrease the permeability of the cell membrane and thus restore normal conditions.

F. R. Lillie ('12) holds that "the action of the spermatozoon in fertilization involves two distinct phases, the first of which may be effected before penetration and brings about a sudden and marked increase in permeability of the egg membrane; the second, which follows after penetration, consists essentially in the establishment of normal interchange between nucleus and cytoplasm, and consequently normal regulation of all the activities of the cell." More recently ('13) he has put forth a new view based upon the reactions of spermatozoa to substances secreted by the ova. He concludes that the 'lysin' which causes cytolysis is contained in the egg, not in the sperm, as Loeb thought; "if cytolysis is involved it is a case of autocytolysis."

My experiments on the giant polar bodies of Crepidula show that changes in the cortical layer which prevent the entrance of a second spermatozoon take place very rapidly over the entire

egg, but that the spermatozoon which enters does not cause any portion of the egg to develop except the cell in which it lies. Although the spermatozoon enters the egg of Crepidula about three hours before the formation of the second polar body the influence of the spermatozoon on the egg protoplasm during this time is not sufficient to start development in the second polar body even though it may contain the greater part of the egg substance. This indicates that the second factor concerned in the process of normal fertilization is not to be found in the diffusion through the egg of some chemical substance carried in by the spermatozoon, but rather in some non-diffusable substance, probably an organic structure.

Long ago Boveri ('87) showed that under certain circumstances the egg of Ascaris may divide at the first cleavage so that half of the egg nucleus passes into each daughter cell while the sperm nucleus does not divide, but goes entire into one of the first two cells. Such a condition he called 'partial fertilization,' and in such cases he found that both halves of the egg develop, thus showing that the activating influence of the spermatozoon has affected both halves. Since in this case the centrosome is the only structure derived from the spermatozoon which is known to go into both cleavage cells he reached his well known conclusion that the essential thing in fertilization is the addition of a centrosome to the egg cell.

It is possible of course that other as yet unrecognized structures are introduced by the spermatozoon and serve to activate the egg. Meves ('11) found that the spermatozoon of Ascaris introduces into the egg a number of coarse granules, the 'plastochondria,' which he thinks unite with similar granules in the egg and are then distributed to the cleavage cells. However, in one of the Echinids he finds that the large granule or 'plastosome' which is derived from the middle-piece of the spermatozoon goes into one only of the first two cleavage cells and yet both develop. I have found that the granules in the eggs of gastropods and ascidians which are presumably identical with 'plastosomes' or 'mitochondria' may be distributed very unequally to the first two cleavage cells without interfering with

the further division of both cells, and there is no evidence whatever that the activating influence of the spermatozoon is due to these granules.

On the other hand many investigators have held that fertilization is essentially a chemical process and that the activation of the egg depends upon the introduction by the spermatozoon of certain chemical substances which diffuse throughout the egg. The observations recorded in this paper indicate that the second or internal factor in normal fertilization is a non-diffusible substance which is introduced by the spermatozoon, and they strongly suggest, though they do not prove, that this factor is the sperm centrosome, a position which Boveri has long maintained and which I have hitherto contested.

5. Cleavage of eggs centrifuged during maturation stages (figs. 33 to 58, 75, 105)

If centrifuging ceases long enough before cleavage begins to allow a return of the egg substances to their usual positions, the cleavage will be absolutely normal, irrespective of where the polar bodies may have been formed. Thus in figures 35 to 44, 47, 49 to 54 the cleavage is proceeding in a wholly normal manner although one or both of the polar bodies were formed at a distance from the animal pole, and in some instances even at the vegetal pole (figs. 35, 36, 38, 39, 44, 105). In figure 40 the effects of centrifuging persisted throughout the first cleavage so that one of the daughter cells contains more cytoplasm and less yolk than the other one, but such eggs may develop into normal embryos, as I have shown elsewhere ('12). Even in such eggs as those shown in figures 44, 45, 49 in which the volume of the 'polar body' may be greater than that of the 'egg,' the cleavage of the latter is perfectly normal except for the smaller size of the blastomeres formed and for alterations in the relative quantities of cytoplasm and yolk. In figure 44 the giant polar body was formed at the vegetal pole which is still marked by the protruding 'yolk lobe;' in figure 45 the giant polar body lies at the animal pole and contains almost all the

yolk; and yet in these two cases, in which the polar bodies lie at opposite poles, the cleavage of the egg up to the 4-cell stage is normal except for the relative amounts of yolk and cytoplasm. The same thing is shown in the later stages of cleavage shown in figures 47, 49 to 54; in spite of the fact that one or both of the polar bodies were formed at a distance from the animal pole, every cleavage takes place in a perfectly normal manner except for slight differences in the relative amounts of cytoplasm and yolk.

Figure 48 is an abnormal egg in which the ectodermal pole lies about midway between the first and second polar bodies; the abnormal cleavage of this egg is probably due to an unequal distribution of cytoplasm and yolk to the first two blastomeres and to a partial separation of one of the blastomeres from the others.

Figures 55 to 57 represent eggs which were centrifuged during the second maturation division, a giant polar body being formed in each case, and in which the cleavage is more or less abnormal. In figure 55, which corresponds to a 24-cell stage, the cleavage is normal in one half (left) of the egg, but abnormal in the other half (right); the polarity in these two halves differs, the ectodermal pole being above in the left half and at the right margin in the right half. In figure 56 four macromeres may be recognized, each of which has produced one micromere, but owing to the partial separation of these macromeres the chief axes are not parallel in the four quadrants; in all probabilities the normal polarity of each macromere is unchanged, but in the process of separation it has been twisted out of its normal relation to the other macromeres. Figure 57 is a 12-cell stage in which there has been a partial separation of two of the macromeres from the other two and in which there is an abnormal distribution of cytoplasm and yolk; the abnormalities of the cleavage are referable to these two factors.

Figure 105 is a 22 to 24-cell stage of an egg which was centrifuged for $2\frac{1}{2}$ hours during the maturation divisions and was then allowed to develop for 18 hours longer. Both polar bodies, one of them large and containing a spindle, lie at the vegetal

pole at the lower side in the figure. The probable identity of the cleavage cells is indicated by the labeling and the arrows.

On the whole then it may be concluded that changes in the maturation pole induced by centrifuging have no lasting influence on the polarity of the egg and do not modify the normal type of cleavage; such modifications of cleavage as do occur may be attributed to, (1) the prevention of the return of nuclei and cytoplasm to the animal pole by long continued centrifuging or by the formation of division walls, (2) abnormal distribution of cytoplasm and yolk to the first two blastomeres and the permanent separation of these substances by partition walls, or (3) the partial separation of blastomeres of the 2-cell or 4-cell stages.

6. The maturation pole does not determine the animal pole of the egg nor the ectodermal pole of the embryo (figs. 35 to 58)

Probably in all animals the polar bodies are formed at the animal pole of the egg and the latter becomes the ectodermal pole of the embryo. However these experiments prove that the polar bodies may be forced to form at any point whatever on the surface of the egg without changing in the least the location of the animal or ectodermal pole. Thus in figures 35 to 41, which represent eggs which were centrifuged for 4 hours and fixed 6 hours later, the original animal pole is clearly indicated by the position of cytoplasm and nuclei and yet one or both of the polar bodies were extruded at a distance from this pole and afterward cytoplasm and nuclei returned to the original animal pole. In figures 47 to 54 the center of the plate of micromeres (ectomeres) is the ectodermal pole and yet this lies some distance from one or both of the polar bodies.

The evidence that it is the maturation pole and not the animal pole which has been moved in these eggs is the following: 1) The polar bodies are larger than normal, showing that they were formed during centrifuging; many of them contain cytoplasm and oil, thus proving that they were formed at the centripetal pole; it is certain that maturation spindles in the early stages may be

centrifuged away from the animal pole (figs. 19 to 21). 2) After centrifuging, the eggs were kept in normal condition from 6 to 24 hours, during which time the cytoplasm and nuclei moved to their present positions; the fact that this movement takes place under normal conditions indicates that it is a movement back to the normal animal pole. 3) The most important evidence that the maturation pole and not the ectodermal pole is shifted by centrifuging is to be found in those cases in which one polar body lies at the ectodermal pole and the other is more or less distant from it. Such a condition is shown in figures 33, 35, 41, 47, 54, 58. In these cases the polar body lying at the ectodermal pole is quite normal in size and appearance, whereas the other polar body is much larger than normal. The plain import of this is that a normal first polar body was formed at the animal pole before the egg was centrifuged, that an abnormally large second polar was formed at the pole to which the cytoplasm and the spindle were displaced and that the cytoplasm and the germ nuclei have moved back to the animal pole after the eggs were removed from the centrifuge. The animal pole is thus marked by the first polar body and though the second polar body may be formed far from this pole it does not change the original polarity of the egg, nor of the embryo which develops from it. These facts constitute, I think, indisputable evidence that *the polarity of the egg and embryo is not the result of the formation of polar bodies at a particular point, but rather this polarity of the egg antedates the maturation and under normal conditions is the cause of the location of the maturation spindles and polar bodies as well as of the ectodermal pole. The maturation pole does not necessarily coincide with the animal pole of the egg, nor does it determine the ectodermal pole of the embryo.*

7. Results of centrifuging after maturation and before the first cleavage (figs. 28 to 30, 59 to 62)

In normal eggs cytoplasm continues to segregate at the animal pole and yolk at the vegetal pole throughout the periods of maturation, fertilization and early cleavage. The egg nucleus and centrosphere lie close to the animal pole and consequently

in a cytoplasmic field, while the sperm nucleus and centrosphere approach the animal pole through the yolk in the vegetal hemisphere of the egg. Other things being equal, the sizes of these germ nuclei and centrospheres depend upon the volume of cytoplasm in which they lie; consequently in normal eggs the egg nucleus and centrosphere are larger than those of the sperm (fig. 28). If eggs are centrifuged in the anaphase or telophase of the second maturation division, both egg and sperm nuclei remain unmoved while yolk may be driven to the animal pole and cytoplasm to the vegetal pole. Under these circumstances the egg nucleus comes to lie in a field of yolk and remains correspondingly small, while the sperm nucleus lies in the cytoplasm and grows large (figs. 23 to 25, 29, 30). In the earlier phases of their growth both egg and sperm nuclei are moved but little by centrifugal force (fig. 59); in later stages both nuclei move more freely (figs. 60 to 62). This is probably due to the fact that in the earlier phases mitotic fibers still bind the nuclei to the surface layer, whereas in later stages these relax.

Even after the germ nuclei have become quite large they may occasionally be seen to be held by a cytoplasmic framework, which connects the egg nucleus to the animal pole, as in figure 60, and which prevents the free movement of the nuclei so that the latter become stretched and distorted under centrifugal force (figs. 60, 61). Sometimes strands of this framework may be seen running through all the zones of the centrifuged egg (fig. 60). On the other hand, if centrifuging continues for a long time the strands become less evident and the nuclei again assume a spherical form (fig. 62), as is also the case when centrifuging ceases. In all these cases nuclei and cytoplasm come back again to the animal pole after centrifuging. This movement takes place especially during mitosis, which probably indicates that this orienting framework is stronger or more active during mitosis than during resting stages.

III. RESULTS OF CENTRIFUGING DURING CLEAVAGE STAGES

An extended study has been made of eggs that were centrifuged at various stages during the first four cleavage periods. Some of these results, which bear on the relation of cell-size to nuclear-size, have appeared in a previous publication (Conklin, '12). In this place we shall consider only the bearings of these experiments on the polarity and pattern of organization of the egg.

1. Modifications of first and second cleavages: equatorial cleavages (figs. 63 to 82, 123)

In normal eggs of Crepidula the first two cleavages are meridional and nearly equal and they are followed by three very unequal cleavages by which three sets of micromeres (ectomeres) are cut off at the animal pole from the large macromeres at the vegetal pole. The direction of every cleavage and its equality or inequality depend upon the direction and position of the mitotic figure and this is controlled by many factors among which the most important are the axes of nucleus and centrosome before division and the relation of these to the polarity and structure of the cytoplasm. At the close of every cleavage (telophase) the centrosphere, nucleus and cytoplasm rotate in each daughter cell in such a manner as to bring the centrosphere to the free border of the cell and as near as possible to the animal pole (Conklin '98, '02).

The mitotic spindles of the first and second cleavages can be moved from their normal positions by strong centrifugal force, though they are sometimes bent and distorted as a result of this and the asters or centrospheres are usually elongated toward their original positions, thus indicating that they are still connected in some way to those positions (figs. 63 to 65).

The entire spindle may be moved from its normal position or one pole may be moved and the other remain relatively stationary. Consequently the spindle may be turned into any axis. In figures 63 and 64 the entire spindle has been moved toward the vegetal pole, the centrospheres stretching back toward their

normal positions; in figure 65 one pole of the spindle has been displaced more than the other one and the spindle is therefore oblique to the chief axis of the egg; in figures 66 *et seq.* the spindle was moved into the chief axis and the resulting cleavage is nearly equatorial in position.

Equatorial cleavages are of particular interest since they afford an opportunity of studying critically the polarity of the egg and the potency of its different parts. When the first (or second) cleavage is changed from a meridional to an equatorial one there is a totally new collocation of nuclei and cytoplasmic substances with respect to the original axis and poles of the egg. Nevertheless, the original polarity is preserved almost unchanged or if modified at all is subsequently restored. In the cell below the equator, cytoplasm, centrosphere and nucleus move during telokinesis to the free surface and as near as possible to the animal pole; in the cell above the equator a similar movement is limited apparently by some attraction on the part of the protoplasm of the lower cell so that cytoplasm, centrosphere and nucleus come to occupy a position more or less intermediate between the lower cell and the animal pole (figs. 67 to 73).

If an egg in which the first cleavage was equatorial is freed from pressure and permitted to develop, the second cleavage will be meridional (figs. 71, 72, 75 to 78) and each of the four macromeres thus formed will give rise to three sets of micromeres (ectomeres) precisely as in a normal egg, except that the micromeres of the cells below the equator are not able to reach the animal pole though they move as far as possible in that direction (figs. 77 to 81). Figure 123 represents an egg in which the second cleavage was equatorial in position. The four macromeres have given off the first set of micromeres (*1a–1d*) and are in process of forming the second set (*2a–2d*); the positions of the spindles and of the cleavage cells are somewhat abnormal in most of the cells. Whatever the factors are which in normal eggs determine the equality of the first two cleavages and the inequality of succeeding cleavages, as well as the polarity of egg and cleavage cells, these factors are still present in centrifuged eggs in which the first or second cleavage

was an equatorial one. The internal organization which determines the position of spindles and the size and position of daughter cells is still present and active, however much it may have been distorted or disturbed.

But although this organization persists in centrifuged eggs, it does not persist as a constant structure which is unaffected by the manner in which it is cut by successive cleavages. If the organization of the egg were antecedent to the cleavage and were in nowise changed by the way in which it is cut by the cleavage furrows, the cell which is formed below the equator should give rise to no ectomeres and the one above the equator should produce all of the ectomeres, and all of these should lie at the animal pole. But this does not happen, the cell below the equator gives rise to its normal number of ectomeres just as the one above the equator does. The dislocation of the first or second cleavage does not change the differential character of the following cleavages.

2. Results of centrifuging during resting stages between first and second and second and third cleavages (figs. 83 to 92, 95, 96)

All cell constituents may be dislocated more readily by centrifugal force during periods of interkinesis than during mitosis, consequently when eggs are centrifuged in resting stages between cleavages, not only are yolk and cytoplasm displaced, but also nuclei and centrospheres. In figures 83 to 92 are shown eggs which were centrifuged in various axes in the 2-cell stage; figures 95 and 96 were centrifuged in different axes in the 4-cell stage. The polar bodies mark the animal pole and in every egg shown the different cell constituents are more or less displaced from their normal positions. But the manner of this displacement shows clearly that these constituents are not free to stratify according to their specific weights. While the major portion of the yolk goes to the centrifugal pole and of cytopalsm to the centripetal pole the boundary between these is not a plane surface, but there are 'lanes' or projections of cytoplasm which remain connected with the animal pole even though most

of the yolk may have been driven to this pole and most of the cytoplasm to the opposite pole (figs. 83 to 91).

Similarly, resting nuclei and centrospheres do not move freely through the cell under the influence of centrifugal force, but are evidently limited in their movements by attachments similar to, but less strong than, the astral radiations of the mitotic figure. The centrosome is always closely attached to the chromosomes, the centrosphere to the resting nucleus, and it is very difficult to separate the two completely. Furthermore, both centrosome and centrosphere are attached by astral radiations to a free surface of the cell and in the telophase of the division this place of attachment is carried as near as possible to the animal pole. Consequently, throughout the resting period the centrospheres are attached on one side to the nucleus and on the other to the free border of the cell lying nearest the animal pole; these attachments persist even in centrifuged eggs and although nucleus and centrosphere may be forced away from the animal pole side of the cell, these attachments are not destroyed, but are merely stretched, as is shown by the lane of cytoplasm leading toward the animal pole, by the elongation of the centrosphere in this direction and by the return of centrosphere and nucleus to their normal positions after centrifuging (figs. 87 to 90). In figures 84 and 85 the axis of centrifuging was parallel with the first cleavage plane and nearly at right angles to the egg axis; in figures 83, 86, 87, 89, 90, 92 the axis of centrifuging was in the chief axis of the egg, the animal pole being centrifugal and the vegetal pole centripetal; in figures 88, 91, 96 the axis of centrifuging was at right angles to the first cleavage plane and to the egg axis; in figures 95, 97, 98 it was at right angles to the second cleavage plane and to the egg axis. These axes of centrifuging are all the principal axes of the egg in which an abnormal distribution of cell substances can be brought about, and yet in every case the centrospheres lie between the nucleus and the animal pole and in some instances the strands connecting these can be clearly seen (figs. 87 to 90, 95 to 98). Figure 86 is especially interesting because the mid-body (*MB*) and spindle remnants are present as well as the nuclei and centrospheres.

The mid-body has remained unmoved by the centrifuging, but the nuclei were carried nearly to the vegetal pole, the centrospheres lie on the animal pole side of the nuclei and the spindle remnant in each cell still connects the mid-body and nucleus, though in this case the nuclei have been moved from the animal to the vegetal pole and the spindle remnant has been bent through an angle of nearly 180°. Figure 92 is especially interesting because one of the two cells contains two nuclei and one centrosphere, while the other contains a centrosphere but no nucleus. Evidently this centrosphere was separated from its nucleus before the division of the cell body began; the cell constriction began at the vegetal pole rather than at the animal pole because cell constrictions always begin on that side of a cell where the cytoplasm is most abundant.

3. Later cleavages of eggs centrifuged in the two or four cell stages (figs. 104, 107 to 112)

In most cases cell substances slowly come back to their normal positions after centrifuging and subsequent cleavages are quite normal; but if the division of the cell body is suppressed or if the macromeres are separated or dislocated, the later cleavages are quite abnormal. Such cases are shown in figures 104, 107 to 112. In figure 104 the second cleavage furrow was suppressed in the right half and at the third cleavage a large protoplasmic cell was formed at the animal pole; the cleavage in the other half of the egg is absolutely normal. In figures 107 and 108 the second cleavage furrow was suppressed in one or both macromeres and as a result some of the later cleavage cells contain multiple nuclei or tetrasters, and are more or less abnormal in position and time of division. Nevertheless, every cell may be identified with a corresponding cell of the normal egg. In figures 109 and 111 the second cleavage was rendered quite unequal, but the following cleavages were very nearly normal except that some of the cells of figure 111 contain multiple nuclei. In figures 110 and 112 the four macromeres were dislocated, with the result that the micromeres form two separate groups; how-

ever, the micromeres formed from each macromere are normal in number and relative position as is best shown in figure 110. In none of these eggs is there any evidence that the polarity or pattern of organization has been changed in any quadrant, though the relations of the different quadrants to one another is changed.

4. Results of centrifuging during the third and fourth cleavages (figs. 97 to 104, 113 to 122)

Centrifuging during the third and fourth cleavages is of especial interest because these cleavages are under normal conditions plainly differential and lead to the formation of the first and second groups of micromeres (ectomeres). In this case as in every other one, the cytoplasm and yolk may be moved more easily than the mitotic figure or the resting nucleus and centrospheres. In figures 97 to 102 eggs are shown which were centrifuged during the third cleavage mitosis; in figures 97 and 98 the axis of centrifuging was at right angles to the egg axis and the fibers connecting the upper pole of the spindles with the animal pole are clearly shown in those cells in which yolk was forced to the animal pole. A later condition of such an egg is shown in figure 100, in which the micromeres *1c* and *1d* were formed some distance from the animal pole owing to the fact that yolk was forced to that pole in the macromeres *1C* and *1D*. In figure 99 the axis of centrifuging was in the direction of the egg axis, the animal pole being centrifuged in position; consequently yolk was driven to the animal pole and cytoplasm to the vegetal one. The further development of such an egg is shown in figures 101 and 102 in which the first set of micromeres (*1a–1d*) are yolk laden and much larger than normal. Figure 102 is especially instructive because it shows the second set of micromeres (*2a–2d*) being formed in a manner entirely normal in quadrants *B* and *C* although the size and contents of the first set of micromeres (*1b*, *1c*) was very abnormal in these quadrants. Furthermore, the orientation of the spindles in the cells *1b*, *1c* shows that a small protoplasmic cell will be formed at the upper pole of this spindle, a large yolk cell at the lower pole, which is just

the reverse of what takes place in normal eggs. This condition may be explained by assuming that *the upper pole of the spindle maintains its normal position by virtue of its attachment to the cell surface at the animal pole, whereas the lower pole of the spindle is relatively free. This condition obtains in practically all stages of maturation and cleavage and it explains one of the most perplexing problems regarding the orientation of the spindle. The attachment of the spindle to the cell surface at the aniaml pole side of the cell is always stronger and more persistent than its attachment at any other point. This is probably due to the greater concentration of spongioplasm at the animal pole.*

The egg shown in figure 103 was centrifuged after the completion of the third cleavage. The micromeres are entirely normal and since they contain no yolk their contents are not displaced, but in the cells *1C* and *1D* the yolk is thrown to the animal pole and the protoplasm is correspondingly displaced. In every cell it is plain that the centrospheres have been displaced least of all the cell constituents.

Figures 113 to 122 represent eggs which were centrifuged for 5 hours in the 4–8 cell stage; the axis of centrifuging was in the chief axis of the egg, the animal pole being centrifuged in position. In all cases the cytoplasm and nuclei or mitotic figures of the macromeres were carried through these cells to the vegetal pole and were kept in this position until one or more sets of protoplasmic micromeres had been formed at the vegetal pole. Figures 113 to 118 were fixed immediately after centrifuging; figures 119 to 122, 5 hours later. Figures 113 to 116 are viewed from the vegetal pole, the polar bodies being shown in dotted outline on the farther side of the egg; figures 117 and 118 represent the same egg, the former showing the cells at the animal pole, the latter those at the vegetal pole. Figures 119 to 122 are viewed from the animal pole, the cells at that pole being shown in heavy outline, while those at the vegetal pole are shown in light or dotted outlines. In figures 113 two of the cells, *A* and *D*, have given off near the vegetal pole small cells containing protoplasm and oil, and similar cells are in process of being formed from the cells *B* and *C*. Figure 115 shows four

protoplasmic cells at the vegetal pole, each of which is dividing or has just divided, while the four macromeres are giving off a second set of small protoplasmic cells at this pole. It is probable that these small cells are micromeres (ectomeres) of the first and second quartets. Figures 114, 116 to 122 were centrifuged after the formation of the first quartet at the animal pole, which is shown in faint or dotted outline in figures 114, 116, 118; in figure 114 four protoplasmic cells, which probably represent the second quartet, lie at the vegetal pole; in figure 116 the second quartet cells are subdividing and a third quartet is being formed; in figure 117 are shown at the animal pole eight cells, four 'centrals' and four 'turrets,' derived from the first quartet, while twelve cells of the second and third quartets are shown in faint outline at the vegetal pole; these twelve cells, which represent eight cells of the second and four of the third quartet, are shown in figure 118 as they are seen through the egg.

If the small cells formed at the vegetal pole in the preceding figures are really micromeres (ectomeres) it should be possible to allow the first quartet to form normally at the animal pole, to then force the second quartet to form at the vegetal pole, and finally to allow the third quartet to come back and form at the animal pole. This is just what has happened in one or more quadrants of the eggs shown in figures 119, 121, 122; in these three eggs the first quartet, which has now subdivided into four 'central' and four 'turret' cells, lies at the animal pole; the second quartet, each cell of which has subdivided, lies in the furrows between the macromeres on the vegetal side of the egg, while in figures 121 and 122 the third quartet has formed outside the first quartet on the animal side of the egg. There can be little doubt, from the manner and time of division of the macromeres and micromeres in these eggs, that the micromeres on the vegetal side of the egg are typical second quartet cells except in respect to their position. On the other hand in figure 120 the first quartet was formed normally at the animal pole, but centrifuging was not sufficiently strong to carry the nuclei or mitotic figures for the fourth cleavage clear through the macromeres to the vegetal pole; as a result the fourth cleavage was a

nearly equal one, the second quartet cells (*2a–2d*), if they may be called such, being large and full of yolk.

5. The potency of substances, regions and blastomeres of centrifuged eggs

From the preceding account it is evident that the fate of any cell in development is not determined by the amount of yolk or cytoplasm which it contains. Typically these two substances are divided equally to the first four blastomeres, but if all of the yolk is thrown into two of these blastomeres and most of the cytoplasm into the other two the ensuing development may be nearly normal.

There is no evidence that the differentiation of a cell is determined by one daughter nucleus or centrosome being different from the other one, for by means of centrifugal force the spindle of a differential cleavage, such as the third, may be forced to take a position so that the micromere will be formed at the lower pole of the spindle instead of the upper pole as normally, or it may be forced into an equatorial position so that the cleavage is into two macromeres and is not differential; in short the micromere may be formed at either pole of the spindle or at neither pole, depending upon its position.

The fate of a cell is not merely a 'function of its position,' as Driesch maintained, but it is in the main a function of its differentiation at the time of its formation; this differentiation depends primarily upon the stage in development at which the cell has arrived and only secondarily upon the direction of cleavage and the position of the blastomere.

Whatever the position, direction or differential character of the first two cleavages may be, the three following cleavages, if freed from outer force, are very unequal, giving rise to three micromeres (ectomeres) at the animal pole side of each cell. These differential cleavages can not occur before the third nuclear division and if at the first or second cleavage a small cell is forced to form at the animal pole it behaves like a macromere and not like a micromere. On the other hand if a third cleavage plane

is forced into a meridional position so that eight macromeres are formed, each of these will give rise to three micromeres, just as each of the four macromeres does in the normal egg. If conversely the second cleavage furrow is suppressed, but the nuclear division is not, each of the two daughter nuclei in each macromere may divide in such a way as to give off the regular number of micromeres from these two macromeres.

All this proves that the formation of micromeres at the third cleavage is not due to the segregation of a peculiar 'micromere substance' at the animal pole, for in whatever plane the first two cleavages may divide the egg or in whatever axis the egg substances may be displaced by centrifugal force, each macromere, if freed from external pressure, still give rise to a typical micromere at the third cleavage. *The localization and orientation of the mitotic spindle, which determines the size and position of the cleavage cells depends upon the viscid spongioplasm or kinoplasm which connects nucleus and centrosphere with one another and with the peripheral layers rather than upon the more movable constituents of the cell; and furthermore, since the orientation of the spindle differs in successive cleavages in a characteristic manner, the orientation of this viscid protoplasm must also differ.*

Consequently, we may conclude that there is an inherent collocation of spongioplasm in every cell which determines the formation of micromeres at the third cleavage, and this does not occur before the third cleavage, since the differentiation of spongioplasm has not proceeded far enough at the first and second cleavages to make possible the formation of micromeres.

I have shown elsewhere ('12) that if a blastomere is isolated in the 2-cell stage, the second cleavage is normal and gives rise to two typical macromeres; if one is isolated in the 4-cell stage, the three following divisions of that cell produce micromeres just as in a normal egg. But no normal micromere can ever be gotten from a cell before the period of the third cleavage. In short, the organization of the kinoplasm in a blastomere of the 4-cell stage differs in some essential way from that in an earlier or later stage, —it has reached a certain peculiar stage of differentiation. Direct observation of eggs in various stages from the time of fer-

tilization up to the period when all the micromeres are formed shows conclusively that one feature of this progressive differentiation of the blastomeres consists in the continuous segregation of cytoplasm at the animal pole and of yolk at the vegetal pole. Before cleavage begins there is a very small area of pure cytoplasm at the animal pole and the entire cell stains with cytoplasmic stains, thus indicating that the segregation of cytoplasm and yolk at the two poles is far from complete; at the 4-cell stage and still more at the 8, 12 and 20-cell stages this segregation is much more complete, the area of cytoplasm at the animal pole is increasingly large and the yolk area stains but little with cytoplasmic stains.

Nevertheless, the differentiation which leads to the formation of micromeres does not depend upon this segregation only, for if the segregation of yolk and cytoplasm is brought about by centrifugal force at the 1 or 2-cell stage it never leads to the formation of micromeres. Even if almost all of the cytoplasm is thrown into two of the cells at the second cleavage and all of the yolk into the other two, each of these four cells gives rise at the third cleavage to a typical micromere. It is evident therefore that micromere formation depends on something other than the segregation of cytoplasm at the animal pole and of yolk at the vegetal pole.

The size of a micromere is fairly constant and is within limits independent of the size of the macromere from which it comes. This is probably due to the fact that the upper pole of the spindle lies at a constant distance from the animal pole. If this distance is forcibly increased or decreased the size of the resulting micromere may be increased or decreased, and if these spindles are turned into a horizontal position the third cleavage planes may be meridional, thus giving rise to eight macromeres, each of which may then give off three micromeres.

This case shows clearly that the differentiation of a blastomere is not due to the differentiation of its nucleus, nor is it wholly due to the position of the cleavage plane, but rather it is caused by a progressive change in the spongioplasm, which change is normally associated with certain mitoses. If the mitoses go on, but the division of the cell body is halted the

differentiation of the spongioplasm proceeds at least for a time, but if the mitoses are stopped the differentiation of the spongioplasm is also stopped.

There is no indication that the nucleus is undergoing differentiation during cleavage; the nucleus in a micromere is evidently of the same character as its sister in the macromere, as is proved by those cases in which the third cleavage spindles are forced into an equatorial position, thus giving rise to eight macromeres; the daughter cells in this case are all macromeres in that each gives off three micromeres, and the nucleus which would have gone into a micromere under normal conditions now goes into a macromere without in any way changing its future differentiations. In short the nuclei of macromeres and micromeres are not differentiated at the time of their formation, but may be thrown about, as Driesch has said, 'like balls in a pile' without changing the fate of any of the cells into which they go.

It is thus possible to show that the differentiation of a blastomere does not depend upon the differentiation of its nucleus nor does it depend entirely upon the segregation of cytoplasm and yolk, nor upon the direction or position of the cleavage plane. After differentiation and localization of cytoplasmic substances has already occurred the direction of cleavage is an important factor, but not before.

IV. GENERAL CONCLUSIONS

1. The nature and causes of cell polarity

Polar differentiation, or more briefly polarity, may be defined as the condition of having unlike poles, particularly in the chief axis of a body, while symmetry is the condition of having like poles in certain axes. The polarity of an entire organism, or of any of its parts, is an expression of the relative positions with respect to the chief axis of subordinate parts having different structures and functions; in this sense it is customary to speak of the polarity of organisms, organs, cells, nuclei, etc., but there is no evidence that the polarity of the entire organism is the resultant of the polarities of its constituent parts, as is the case with magnetic polarity.

Organic polarity may be viewed from the standpoint of structure or of function; neither of these aspects is complete in itself and neither is at variance with the other but the two are complementary. In a series of important papers ('11–'15) and in a recent book ('16) Child has announced that the polarity of various adult organisms and of certain eggs and embryos is shown physiologically as a gradient in the rate of metabolism from one pole to the other. But in spite of this important discovery polarity can not be looked upon as a physiological process merely; there must be a material, structural basis for such a gradient of metabolism; furthermore it has not been demonstrated that physiological differentiations are the causes of morphological ones, for although functional changes are often more readily visible than structural ones, there is every reason to think that structure and function are inseparable in living organisms and that neither is the cause of the other. In this paper attention is devoted largely to the morphological aspects of polarity, but it is not to be assumed therefore that the author considers the physiological aspects as negligible.

The polarity of the egg cell is the earliest recognizable and most fundamental differentiation of morphogenesis; it is the chief factor in determining the localization of developmental processes, such as the segregation of different oöplasmic substances and of specific physiological activities, the orientation of mitotic figures and cleavage planes, and finally the determination of the polarity and symmetry of the embryo and of the adult. In short the polarity of the organism in the one-celled stage of development is the chief condition and cause of the polarity of all later stages. In many animals the polar differentiation of the egg may be recognized even in the stages of its development in the ovary and it is probable that in all cases such polar differentiation exists at this time.

When first recognizable this differentiation usually consists in the eccentricity of the nucleus and centrosome and of the greater part of the cytoplasm toward one pole of the egg and the greater accumulation of yolk at the other pole, the former being known as the animal pole and the latter as the vegetal. Dur-

ing the period of maturation, preceding or accompanying fertilization, two minute cells, the polar bodies, are formed at the animal pole of the egg, and in all animals from sponges to mammals the animal pole gives rise to the ectoderm and the vegetal pole to the endoderm of the embryo.

But while the polarity of the embryo as a whole corresponds directly to the polarity of the egg from which it develops the axes of the constituent cells of the embryo are shifted in successive cell generations so as to form all possible angles with the chief axis of the embryo. The axis of a cell is usually marked out by the line passing through the center of the resting nucleus and centrosome, this being the 'cell axis' (Van Benedan '83, Heidenhain '94). In cleavage cells the resting centrosome, or centrosphere, comes to lie at the free border of the cell at a point as near as possible to the animal pole, consequently in cells lying near the animal pole the cell axis is nearly parallel with the chief axis of the egg, but in cells which lie near the equator of the egg the cell axis may be nearly at right angles to the egg axis and in cells which lie near the vegetal pole the cell axis may be nearly the reverse of the egg axis. As long as the cleavage cells are relatively large it is possible to see that the resting centrosomes always lie at that point of the free surface of the cell which is nearest the animal pole, but when these cells become very small it is no longer possible to see that the centrosomes are turned toward the animal pole although they always lie at the free border of the cell. Similarly in tissue cells it can be seen in many cases that the centrosomes lie at the free border of the cell (Heidenhain and Cohn '97), but there is no evidence of their approximation to the original animal pole. The same is true of the various stages in the formation of the germ cells, whether ova or spermatozoa, the centrosomes always lie on the side of the nucleus toward the free border of the cell and away from its attached side, but no approximation of the centrosome to the original animal pole can be traced.

The resting nucleus shows polar differentiation, as was pointed out long ago by Rabl ('85), the pole which lies nearest the centrosphere being known as the 'Pol' or central pole, the opposite

one as the 'Gegenpol' or distal pole. Experiments show that the centrosphere is attached rather firmly to the central pole of the nucleus and however much the relative positions of the different parts of the cell may be changed this attachment of centrosphere to nucleus is rarely broken. Within the nucleus the developing chromosomes are aggregated chiefly at the central pole, the achromatin at the distal pole.

The centrosphere also shows a polarity of its own, its chief axis being that in which the daughter centrosomes move apart and form the initial spindle. This initial spindle, or 'netrum' of Boveri ('00), usually lies at right angles to the cell axis and to the preceding spindle axis. But the axis of the fully developed spindle within the cell may differ from that of the initial spindle since there are characteristic movements of the cytoplasm of every cell which transport the spindle into its definitive position. During the last phase of nuclear division (telophase of Heidenhain '94) the daughter centrosomes and nuclei turn back toward the original cell axis, the centrospheres remaining attached to the nuclei at their central poles and moving to that point on the free surface of the cell which is nearest the animal pole; the new cell axes which are thus formed are approximately but not exactly parallel with the old cell axis. In egg cells and cleavage cells the cell axis does not change every time the centrosomes separate in division, but this axis remains relatively constant while the mitotic figures may form any angle with it, but at the close of division the centrosomes and nuclei come back once more into the cell axis. Thus the axis passing through nucleus and centrosome coincides with the cell axis only during the resting stage of the cell.

Although the cell axis is usually marked out by the position of the resting nucleus and centrosphere, it is not entirely dependent upon that position. By pressure or centrifugal force the positions of nuclei and centrospheres may be changed without permanently altering the cell axis, as is shown by the fact that these structures usually come back once more to their normal positions as soon as the pressure is removed. Similarly the position and direction of the mitotic figure and of the resulting cell division

may be changed experimentally without changing the real cell axis. In short the cell polarity persists in the organization of the cytoplasm after the positions of centrospheres, nuclei, mitotic figures and cleavage planes have been changed. But while in such cases the polarity of the cell persists in the cytoplasm, there is evidence that the polarity of the cytoplasm has developed in connection with and in definite relation to the polarity of the nucleus and centrosome.

With regard to symmetry it is known in a few cases, notably cephalopods and insects, that the egg is bilateral even in the ovary; in other cases such as amphibians and ascidians, bilaterality first becomes apparent shortly after fertilization; in still other cases bilaterality does not become evident until later stages of the cleavage or even of the blastula or the gastrula. In the case of sinistral gastropods the inverse symmetry may be traced back in development to inversely symmetrical cleavage of the egg, indeed to the very first cleavage, and it is evident that the causes of this inversion must be present in the egg before cleavage begins (Conklin '03).

In addition to these general axial differentiations of polarity and symmetry other more specific differentiations of regions and substances of the egg exist in some animals. In ascidian eggs the substances which give rise to different organs and tissues, such as the nervous system, the chorda, the caudal muscles, the mesenchyme, the ectodermal and endodermal epithelium are definitely localized and may be clearly distinguished as early as the first cleavage (Conklin ('05). Although these eggs show an unusual degree of differentiation at a very early stage, there are many others in which the 'pattern of localization' is present either before or just after cleavage begins. Among the gastropods generally the first cleavage separates an anterior blastomere (*A B*) from a posterior one (*C D*), the second cleavage divides these into right and left halves. Each of these four blastomeres gives rise to three ectomeres and to a large entomere, while the left posterior cell (*D*) gives rise also to the mesomere (*4d*). Each of these cells produces in later stages a definite portion of the embryo so that the development is, as Wilson has

said, a 'visible mosaic work.' All of these orientations of development find their earliest visible expression in the polar differentiation of the egg; the problem of the causes of these orientations is perhaps the greatest problem of embryogeny.

Causes of cell polarity

Any satisfactory explanation of the causes of polarity and symmetry of cells must be able to explain the following phenomena:

a. The typical localization of substances in cells, such as yolk at the vegetal pole and of cytoplasm and nucleus at the animal pole, together with the typical orientation of spindles, centrospheres and other cell constituents during and after mitosis.

b. The return of all cell substances to their typical positions after they have been displaced if sufficient time and opportunity for this return are given, and if the injury to the cell is not too severe.

It is therefore evident that the cause of polarity in cells is one of the most fundamental problems in the study of the structure and functions of cells and in the processes of differentiation and regulation. The localization of formative substances in eggs determines the localization of the parts of the developing embryo, and the return of these substances to their normal positions when once they have been displaced is a remarkable case of adaptation or regulation in which the organization concerned is merely the polarity of a single cell. Because of the apparent simplicity of this problem of the polarity of the cell, the hope is raised that a thorough analysis of it may throw light on the problems of differentiation and regulation in general.

It is conceivable that the causes of this polarity may be due (1) to electric charges on the colloidal particles of protoplasm, or more particularly on centrospheres and cell membrane, or (2) to external or internal surface tension phenomena, or (3) to a framework of viscid protoplasm which is so elastic or contractile that it recovers its normal form after distortion. The

evidence for or against these possibilities may be considered briefly.

1. Electric polarity: polarity of fused eggs. There is really little or no evidence that cell polarity is of the nature of electric polarity. Neither the entire egg nor any of its parts orients with respect to a constant current passed through the water in which eggs are placed, and none of the constituent parts of a cell is moved or oriented by an electric current passed through the cell itself unless that current is so strong that phenomena of convection occur (see Conklin, '12).

A certain amount of light is thrown upon the nature of the polarity of the egg by the effect on this polarity of the fusion of two or more eggs. If this polarity were the result of electric charges carried by colloidal particles or if it were due to different properties of the cell membrane at the two poles, the polarity of fused eggs should be the resultant of the polarities of its components. On the other hand if the polarity is due to an internal framework of protoplasm which is but slightly miscible with other similar frameworks and which therefore preserves to a great extent its identity, the original polarity of two or more eggs would not be much changed by their fusion. The facts show that the latter is the case.

When eggs of Crepidula are strongly centrifuged they are pressed closely together and frequently adhere to one another. Very rarely they fuse together so that no boundary can be seen between the two. Thus figure 106 represents two eggs which fused together in the 4-cell stage along the plane indicated by the dotted line. Each egg is now in the 8-cell stage and each preserves its original polarity, as is shown by the polar bodies, micromeres centrospheres and nuclei. Although each egg is in the 8-cell stage, the fusion of three pairs of cells reduces the total number of separate cell bodies to 6 macromeres and 7 micromeres. The nuclei and centrospheres of the fused cells are quite distinct and even the cytoplasmic areas are partially separated by a tongue of yolk.

It rarely happens that eggs are fused together in experiments with centrifugal force, and figure 106 represents one of these

rare cases, but it is very easy to cause the fusion of eggs by other means. For example in experiments where eggs are treated with carbonic acid there are many cases of such fusion. Sometimes two eggs are fused as in figure 106, or three, four, or many eggs may be fused into one mass. It is an interesting fact that eggs rarely if ever fuse by their protoplasmic poles, but almost invariably by some portion of the lower hemipshere which contains yolk. There is apparently some peculiarity of the egg surface over the animal hemisphere which prevents its fusing with another egg at this pole. A large number of such eggs which were caused to fuse together at various stages before and during cleavage has been studied, and in every instance the polarity of each constituent egg remains practically unchanged. The ectomeres from different eggs unite in later stages of development into a continuous layer, but there is no indication that the polarity of one cell is changed by its fusion with another cell, as would be true if polarity were due merely to electric charges on colloidal particles or to physical properties of the cell membrane. On the other hand these observations indicate that the polarity of an egg inheres in the organization of its more viscid protoplasm which is but slightly miscible with that of other eggs.

2. Surface tension as a cause of polarity. It is possible that the various constituents of cells are oriented and held in place, or brought back to normal positions if displaced, by surface tension. For example if spindles or centrospheres which are attached to the surface layer are centrifuged strongly, that layer may be indented as in figures 5 and 6 and the surface thereby increased; in such a case surface tension would restore the spherical shape of the cell after centrifuging, and if the asters or spheres still remained attached to the surface they would be drawn back to their normal positions. However, I have never seen the egg surface indented as shown in figures 5 and 6 except in the stage of the first maturation spindle and even at this stage it is unusual. In no other stage figured in this paper is there any such indentation of the cell and yet in every case all cell parts come back if possible to their normal positions

after centrifuging. External surface tension can not therefore be the cause of the normal positions of cell parts or of their return to these positions after centrifuging. Probably internal surface tension between the different constituents of a cell may play a more important part in localization of these constituents, but even this would not account for the persistence of polarity in centrifuged eggs unless there is some portion of the cell which remains unmoved during centrifuging.

Since surface tension increases with decreasing temperature and *vice versa* an attempt was made to determine whether the return to their original positions of cell substances or parts which have been displaced by centrifuging is hastened by lowering the temperature. Eggs in various stages of development were centrifuged for 10 minutes and then placed for varying lengths of time on ice where the temperature was about 2°C., while one control was kept at room temperature (about 20°C.); in another experiment the temperature was raised to about 35°C. That the surface tension of these eggs is increased by lower temperatures is indicated by the fact that eggs are more nearly spherical at lower temperatures than at higher ones, but the results showed conclusively that the return of cell parts to their normal positions took place more rapidly at about 20° than at 2°, thus indicating that this return is not due to internal or external surface tension. It is true that viscosity increases at lower temperatures as well as surface tension, nevertheless it does not prevent the eggs from assuming a spherical form; for these reasons, as well as for those mentioned above, it is evident that neither external nor internal surface tension is the principal cause of the normal location of cell parts nor of their return when once they have been displaced. However it is not denied that internal surface tension may be one of the contributory factors in the return of displaced substances, such as yolk, to their normal positions.

3. Spongioplasmic framework as the cause of polarity. There remains the explanation which has been maintained throughout this paper, namely, that the orientation and localization of cell

parts is due to a framework of more viscid protoplasm and that the return of displaced parts to normal positions is due in the main to the elasticity or contractility of this framework. The evidence in favor of such a view may be summarized as follows:—

a. The substances of the egg of Crepidula are never completely stratified by centrifugal force of from 600 to 2000 times gravity. Of all substances in the egg the yolk and cytoplasm are most completely stratified and yet the boundary between the two is never a plane, as it would be if the substances were free to move according to their specific weights, but the boundary between these substances is an irregular one with 'lanes' or projections of cytoplasm into the yolk. This indicates that while there is a relatively large amount of cytoplasm which is freely movable within the cell, there is a small amount of more viscid substance which penetrates every part of the cell and is especially abundant in nuclei, centrospheres and mitotic figures; this viscid material prevents the complete stratification of cell substances according to their specific weights.

b. In many instances strands of this viscid substance may be seen running through various portions of eggs; such strands are seen most plainly in the mitotic spindles and astral radiations of dividing cells and also in the connections between nuclei and centrospheres and between the latter and the cell surface in dividing cells. These connections may be stretched or bent, but are rarely broken. The fact that when the animal pole of the egg is centrifugal in position the spindle may be stretched or distorted and the surrounding cytoplasm may be forced away, while yolk comes to be densely packed around the spindle, proves that the spindle is not merely the expression of lines of force, like iron filings in a magnetic field, but that it is a relatively persistent structure of a viscid or gelatinous character. The same is true also of resting nuclei and centrospheres and of the strands which connect these to the cell surface (see fig. 60).

c. This viscid material is most abundant in spindles and astral radiations of dividing cells and in nuclei, centrospheres and the connections between these and the cell surface in rest-

ing cells. It serves to hold the fully formed mitotic spindle in a definite position with respect to the cell surface; in early prophases and in resting stages these connections are relaxed so that spindles or nuclei may be moved out of their normal positions, but these connections are not easily destroyed and they always hold the nuclei and centrospheres in the same relative position to the cell surface, however much they may be stretched.

2. The structure of protoplasm

The identification of a more viscid and a more fluid portion of the protoplasm in centrifuged eggs leads to a consideration of the relation of these two constituents of protoplasm to each other and to various cell inclusions; it also raises the question of their relation to the polarity and orientation of development.

It has long been evident that protoplasm is not a homogenous fluid. Dujardin held that 'sarcode' was a "substance glutineuse, parfaitment homogène, elastique, contractile, diaphane. . . . . On n'y distingue absolument aucune trace d'organization, ni fibres, ni membranes, ni apparence de cellulosité." (Quoted from Henneguy 'La Cellule,' p. 31.) Max Schultze, Haeckel, Kühne and many other early observers regarded protoplasm as a fluid owing to phenomena of protoplasmic flowing and of surface tension. Brücke ('61) first contested the possibility of this on *a priori* grounds, holding that a homogeneous fluid would be unable to perform the functions which protoplasm performs, and maintaining that it must have a 'special structure' or 'organization' made up of more liquid or more solid parts among which are the 'smallest living parts' or vital units. All students of protoplasm now agree that it is composed of more fluid and more solid parts, though there is much difference of opinion as to the form of each of these and their relation to each other, as is shown by the various theories on the 'structure of protoplasm.' Different names have been given by authors to the more fluid and the more solid parts of protoplasm as indicated in the following incomplete list:

More fluid part	*More solid part*	*Author*
Ground substance Enchylemma	Reticulum	Heitzmann, 1873 Carnoy, 1883 VanBeneden, 1883
Alveolar Substance	Interalveolar Substance	Bütschli, 1873–1892
Paramitome	Mitome	Flemming, 1882
Hyaloplasm	Spongioplasm	Leydig, 1885
Trophoplasm	Kinoplasm	Strasburger, 1892
Etc.	Etc.	

Some of the earlier students of the cell considered that only one of these substances was 'living,' though they differed as to whether it was the more fluid or the more solid part. Wilson ('00, p. 30) concludes that "we are probably justified in regarding the continuous substance (i.e. spongioplasm, inter-alveolar substance, kinoplasm) as the most constant and active element and that which forms the fundamental basis of the system, transforming itself into granules, drops, fibrillae, or networks in accordance with varying physiological needs." With this opinion I entirely agree.

In addition to these two substances protoplasm contains many other parts, some of which as Wilson suggests are formed probably as differentiations of the spongioplasm, others perhaps as differentiations of the hyaloplasm. Among the substances which are embedded in the protoplasm, but are not a part of it, are the 'inclusions' such as oil, water, yolk, etc.

Lillie ('06, p. 156) says of the protoplasm of the egg of Chaetopterus, "The ground substance is a suitable name for the fluid that contains and suspends all the granules and droplets; if these were imagined removed it would preserve a faithful semblance of the egg. Thus it is regarded as forming the external pellicle and as continuous through the nuclear membrane with the nucleoplasm." Speaking of the vibrations of the microsomes and spherules in living protoplasm, Lillie says, "No one who has studied these movements, as I have done for hours at a time, could believe that the microsomes are nodal points of a network, or are connected by filaments as they appear to be in the best stained sections. One is forced to conclude that they have freedom of movement in all directions, i.e., that they are suspended in a

fluid medium which has no filar, reticular or alveolar structure." Lillie further concludes that "microsomes are the primitive formed elements of the cytoplasm" and that in point of origin they are chromatin particles. The appearance of a reticulum in fixed eggs he holds to be an artifact due to coagulation of a colloidal solution.

It is unquestionably an extremely difficult task to determine with certainty the 'ultimate structure' of a substance which is so changeable in appearance as living protoplasm. There is, however, good reason for believing, as I have attempted to show, that it is composed of a more fluid and a more viscid portion and that while the former may be moved readily by centrifugal force the latter is not so readily moved; also that this more viscid part of the protoplasm holds nuclei and centrospheres in a definite relation to the periphery of the cell and brings parts back to their normal positions when once they have been displaced; in short that the polarity and morphogenetic organization of the egg reside in this more viscid substance, and not in the more fluid medium as Lillie maintains.

Whatever the ultimate structure of this denser portion of the protoplasm of the egg of Crepidula may be, it is certainly not a true fluid, nor is it the more fluid portion of the protoplasm. It does take the form of fibers or strands in the amphiaster of the living egg and these strands anchor the amphiaster to the peripheral layer; in resting stages similar fibers are present between nucleus and centrosphere and between the latter and the periphery of the cell. I suspect that the microsomes of which Lillie speaks are not a part of this denser protoplasm, but that they lie in the more fluid substance, which would account for their freedom of movement and would also explain the fact that the microsomes aggregate to so large an extent in the middle zone of centrifuged eggs. Mitochondria also, in this respect at least, behave as microsomes; in the eggs of mollusks and ascidians they move through the cell under the influence of centrifugal force almost as freely as do yolk spherules and oil drops.

It is customary to explain the polarity or other differentiations of an egg as the result of its organization. But 'organization'

is a general and indefinite term which may include anything from metaphysical entelechies to hard and fast structures. Omitting from consideration all hypothetical causes which are beyond the reach of experimental investigation, we find that polarity, development, regulation or any other vital phenomenon may be regarded from the standpoint of static or of kinetic conditions, of morphological or of physiological causes. Ideally these distinctions are sharp and definite, but they are not so in reality. In a living organism static and kinetic, morphological and physiological conditions are really inseparable.

However, for the sake of clear thinking, it is necessary to form some sort of a mental picture of what is meant by such a phrase as 'the organization of the egg.' On its morphological side the polar organization consists, as I have attempted to show, in a relatively persistent framework of viscid material which is also elastic and contractile so that it tends to resume its normal form when distorted; by this framework nuclei, centrospheres and mitotic figures are bound more or less firmly to the peripheral layer or 'Hautschicht' (Strasburger). There is no good evidence that this viscid material exists in the form of fibers which are definite in number and position. On the other hand its behavior during centrifuging would indicate that the appearance of fibers is due to inclusions which are forced into an otherwise continuous substance; this substance is more abundant and more uniformly continuous at the animal pole than elsewhere in the cell. In normal eggs the presence of large yolk spherules at the vegetal pole gives to this substance a coarse sponge-like texture while the smaller spherules of yolk, oil and enchylemma toward the animal pole give to it a finer alveolar character. This appearance is very evident in good sections of normal eggs, as is shown in the figures of my paper on Karyokinesis and Cytokinesis (Conklin '02), and that this structure is not an artifact, but is normal, is confirmed by the experimental studies of this and of a former paper (Conklin '12).

When yolk is driven to the animal pole by centrifugal force the fluid portion of the cytoplasm and much of the viscid portion are driven away and that which remains is stretched or compressed

into strands or fibers which are not to be regarded as preformed structures. It is clear that the localization of yolk at the vegetal pole in normal eggs and its return to that pole after it has been displaced is due to some differentiation of the spongioplasm at the two poles, for otherwise the yolk might be localized at any pole and would remain wherever it happened to be thrown. In normal eggs the spongioplasm is more abundant at the animal pole than elsewhere, but in centrifuged eggs it may be less abundant at this pole and yet normal conditions may be restored after centrifuging. It is therefore necessary to assume that the spongioplasm differs in some way, perhaps in elasticity or viscosity, at the two poles. If this material is more elastic or more contractile in the region of the animal pole than elsewhere in the cell the localization of cytoplasm and yolk in normal eggs and the return of dislocated substances to their normal positions would find an explanation. Furthermore the connections of centrospheres and nuclei, the orientations of mitotic figures and the progressive localizations of cell substances indicate that the spongioplasm must differ in different regions of the egg and at different stages of development.

The question may well be raised whether the spongioplasm, or more viscid portion of the protoplasm, is not the real formative material, while the cytolymph as well as the oil and yolk are mere inclusions. I have already indicated that it is the more important or indispensable part of the protoplasm, as is shown by experiments with centrifugal force and also by the desiccation of protoplasm in seeds and in certain animals (rotifers, tardigrades, etc.); in both of these cases the fluid within the protoplasm may be largely eliminated without permanently destroying or injuring the protoplasm. It is well known that the fluidity or viscosity of protoplasm depends upon its water content and that this differs under different external conditions and in different stages of the cell cycle. Evidently the colloids in protoplasm may change from gels to sols and *vice versa*. It does not seem wise therefore to identify as protoplasm the gels only. The most convincing work which has yet been done on the physical properties of protoplasm in living cells is that of Kite, Chambers,

et al., by the method of microdissection. Kite ('13) found by this method that the living cytoplasm of the egg of Asterias is an apparently homogeneous and a very viscous gel in which microsomes and globules are suspended. This gel he found to be very elastic so that when portions of it were drawn out with a needle they would at once retract when released. On the other hand he found that the nuclear substance of this egg, with the exception of the nucleolus which is a quite rigid gel, is all in the sol state. In the male germ cells (probably spermatocytes) of insects he found that the cytoplasm and nucleus of the resting cells "are far too rigid to flow or change shape under such experimental treatment. In the dividing cells the spindle fiber is an elastic concentrated thread of nuclear gel and its absorptive power and refractive index are also different from those of the dilute gel in which the spindle fiber is imbedded and from which it cannot be entirely freed. The homogeneous gel in which a telophase spindle is imbedded is so rigid that all the surrounding cytoplasm can be cut away and the spindle and chromosomes show no appreciable change; metaphase, anaphase and telophase spindles can be cut to pieces in Ringer's fluid and the pieces are so rigid that they undergo no change in shape." Finally he concludes "that cell division results primarily from concomitant shrinking and swelling or changes in water holding power of different portions of the cell protoplasm. Many of the structural elements of the mitotic figure separate out of the protoplasm and change in rigidity according to their water content. During the prophase the nuclear substance becomes so soft that movement of the components of the nucleus is affected by flowing of the nuclear gel. The mechanism at the basis of this flowing seems to be a change in the water holding power of the nuclear components."

Chambers ('15) found that "the dissection of the germ cells of insects and of the frog reveals an extreme variability in consistency of their protoplasm, depending probably upon their water content." He also found that "in many egg cells and free living unicellular organisms the surface layer of protoplasm may be decidedly more rigid than the interior." The nucleus of a

living cell consists, according to him, of a gelatinous substance "surprisingly more rigid than the cytoplasm in which it lies." Mitochondria are not persistent structures but "they disappear and reappear and must be merely changes in the physical states of the colloids which compose the cytoplasm."

My own observations and experiments on resting and dividing cells, most of which were made before the publication of the work of Kite and Chambers, lead to essentially similar conclusions. The cytoplasm of the eggs of gasteropods and ascidians is composed of a viscid, elastic, contractile gel in which are included water, oil, yolk, pigment, microsomes, etc. This gel is more rigid at certain phases of the cell cycle than at others depending probably upon its water content. During the resting stage the nuclear contents are in a state of gel, but in the beginning of the prophase the achromatin becomes more fluid. It is quite evident that the nucleus grows by absorbing substance from the cytoplasm. If the nuclear membrane is really a membrane, and there is much evidence that it is, such absorbed substance must enter as a fluid, though once within the nuclear membrane it is converted into a gel. On the other hand when the nucleus reaches the prophase of mitosis much of its contents becomes more fluid and flows out toward the centrosomes where it again gels in the form of astral rays and spindle fibers (Conklin, '02, '10, '12 a, b).

3. Protoplasmic flowing and intracellular movements

Another general phenomenon which is involved in these conclusions is that regarding the nature of protoplasmic flowing and intracellular movements. In the eggs of Crepidula more or less extensive movements of the cell substance take place, as shown in the movements of the maturation spindles to the animal pole, the migration of the sperm nucleus and aster through the egg, the segregation of cytoplasm at the animal pole and of yolk at the vegetal pole, and the movements of metakinesis and telokinesis during cleavage. If the protoplasm is a viscid, elastic, contractile gel how can such movements be explained?

In these movements we may distinguish the active movements of protoplasm which occur in the localization of cytoplasm, nuclei and mitotic figures, and the passive movements of inclusions such as oil, yolk and pigment. These passive movements may be considered first since they are simpler than the active ones. They are plainly of two kinds: 1) inclusions such as pigment, granules, water, etc. may be carried along with protoplasm in its active movements, as in the case of the yellow pigment in the 'mesoplasm' of Cynthia (Conklin '05); 2) by the concentration of protoplasm in certain regions inclusions may be forced out of those regions as in mitotic spindles and asters; similarly the concentration of protoplasm at the animal pole forces yolk to the vegetal pole. Such passive movements are due to active movements of the protoplasm and require no further explanation.

The active movements of protoplasm are more difficult to observe and explain. The denser portion of the protoplasm is highly elastic and contractile, as Kite has shown by direct observation, and its capacity for movement is probably due to this property. Thus the flowing of peripheral protoplasm to the point of entrance of a spermatozoon and the formation there of an entrance cone may be regarded as due to the contraction or concentration of this protoplasm to the point stimulated. Probably the collection of spongioplasm around the sperm centrosome or in the aster of any mitosis is likewise due to the contractility of this substance. The movement of the sperm nucleus and aster toward the animal pole and the segregation there of most of the spongioplasm may be explained in the same way. If the spongioplasm is highly contractile in all directions and concentrates to a point of stimulation these and many other cell activities find a ready explanation. The possibility of such concentration depends of course upon the fact that spongioplasm is not uniformly distributed throughout the cell but that it exists intermingled with other substances and that in its concentration to one point these other substances are displaced. In former papers (Conklin '02, '12) I have dealt at some length with the movements of metakinesis and telokinesis and since I have

nothing new to add to those conclusions I need only say that the flowing movements which I there described may be interpreted as the result of the contractility of the spongioplasm,—which is indeed the original explanation of these movements (Van Beneden '87, Boveri '87).

4. The orientations of development

According to the view here expressed the localizations of spindles and cleavage planes, of nuclei and centrospheres, of cytoplasm and yolk, and indeed the orientation of all developmental processes is associated with the structure and activities of the spongioplasm. In eggs generally cytoplasm becomes concentrated at the animal pole during early stages of development and coincidently yolk is forced away from that pole, probably by contraction of the spongioplasm to the animal pole; nuclei and centrospheres are bound together and are held in a definite relation to the animal pole by strands of spongioplasm; mitotic figures are oriented by means of the framework of spongioplasm and the planes of cleavage are thereby determined.

In all cases the position and direction of the division planes is controlled by the position and direction of the spindle in the later stages of mitosis, the division plane always passing through the equator of the spindle and at right angles to its axis. In normal eggs of Crepidula the first maturation spindle forms in the position previously occupied by the germinal vesicle—a little removed from the surface of the egg. This spindle reaches its maximum length in the metaphase at which time it is about as long as the radius of the egg. In the anaphase the peripheral pole of the spindle comes into close contact with the peripheral layer of protoplasm and at the same time the aster at this pole grows smaller and smaller and is at last completely absorbed into the peripheral layer while coincidently the spindle grows shorter so that when the division wall is formed through the equator of the spindle it cuts off a very small polar body from a relatively enormous egg. On the other hand where both asters are attached to the peripheral layer as in certain cleavages, the

spindle grows longer during the anaphase. If the maturation spindles are prevented from shortening, giant polar bodies are formed since the division plane must pass through the equator of the spindle. If the first cleavage spindle is turned into the chief axis of the egg the cleavage plane is equatorial, instead of meridional as it should be, since the division plane must be at right angles to the spindle axis.

However the initial position and direction of the spindle may differ from its definitive orientation. In several earlier papers on Crepidula ('97, '98, '99, '02) I have shown that the spindle may form out of its definitive position and subsequently be moved into it by the activity of the cytoplasm. Lobes of cytoplasm may indicate where micromeres will form while the newly formed spindles are yet some distance away from these lobes; ultimately one end of a spindle moves into each of these lobes and then the cell division takes place through the equator of the spindle cutting off a micromere from a macromere.

Without doubt the position and direction of a cleavage furrow is determined by the position and direction of the fully developed spindle, but what determines the orientation of the latter? It is sometimes assumed that the orientation of a spindle is determined by yolk or other inclusions, for example that micromeres are formed at the animal pole because yolk is segregated at the vegetal pole and this displaces cytoplasm, nuclei and spindles toward the animal pole,—but this is quite erroneous. In this paper as well as in a former one ('12) I have shown that the pattern of cleavage is more or less independent of the amount of yolk, oil, water or other inclusions contained in a particular egg or blastomere, and Lillie ('06) and Morgan ('10) found this to be true in the eggs which they centrifuged. If the yolk which collects at the centrifugal pole or the oil which collects at the centripetal pole are thrown out of the egg completely the remainder of the egg which contains the nucleus and the material of the middle zone may segment like a normal egg, the first and second cleavages being approximately equal and the subsequent ones unequal, thus giving rise to four macromeres and to three sets of micromeres which form from these. If the yolk is thrown

into one of the first two blastomeres and the lighter substances together with most of the cytoplasm into the other one, each of these blastomeres continues to segment in a normal manner, the second cleavage being approximately equal and the subsequent ones giving rise to macromeres and micromeres as in normal eggs. The formation of macromeres and micromeres therefore does not depend upon the presence of yolk in the former but upon some other factor.

But if the pattern of cleavage is not determined by the cell inclusions it is equally clear that it is not determined by any fixed and unalterable localization of the protoplasm with respect to the cell axes, for if the first cleavage plane is forced to take an equatorial position the second cleavage is meridional and equal and from each of the four cells thus formed micromeres are cut off on the animal pole side as in normal eggs. In short the macromeres which lie at the original animal pole are not the only ones which form micromeres, but even those which were cut off below the equator of the egg also form micromeres. This shows that the pattern of cleavage is not predetermined with reference to the original polarity of the egg. On the other hand eggs which have been subjected to pressure in the direction of the chief axis of the egg at the time of the third cleavage may divide so as to form five, six, seven or eight macromeres and in such cases each of these macromeres gives rise later to three sets of micromeres as if it were a normal macromere. Consequently it cannot be said that the character of the cleavage is determined by an inherited and wholly definite orientation of each succeeding cleavage spindle, for if this were the case, when the third cleavage is rendered equal by pressure, subsequent cleavages should give rise only to second and third sets of micromeres; indeed when the third cleavage is forced to be an equal one the subsequent cleavages occur as if this were an entirely new cleavage which had been intercalated between the typical second and third cleavages.

In conclusion, then, the pattern of the cleavage is dependent upon the position and direction of the spindles and this is determined, not by inclusions, but by the spongioplasm which holds

nuclei, centrospheres and mitotic figures in a definite relation to the cell axes, and which is so elastic that when it is distorted by pressure or centrifugal force it tends to bring parts back to their normal positions. But such an explanation does not explain the thing which we most wish to know, namely what determines the definite, orderly succession of orientations in development. For example, why do the two maturation spindles usually have the same orientation, while all succeeding divisions of the egg alternate in direction? Why are the first and second cleavages in Crepidula equal while subsequent ones are unequal? Why does every cleavage take place normally in a perfectly definite way, which differs from every other cleavage, and give rise to perfectly definite blastomeres which differ from all other blastomeres? If the orientations of development depend upon the spongioplasm does the structure of this spongioplasm change in a definite way from cleavage to cleavage? These are questions which for the present must be left unanswered.

V. SUMMARY AND INDEX

1. If the fertilized but unsegmented eggs of Crepidula plana are subjected to a centrifugal force of approximately 600 times gravity yolk is thrown to the centrifugal pole, oil and other light substances to the centripetal pole, while nucleus, centrosphere and most of the cytoplasm occupy the middle zone between the other two. The relative volumes of these three zones is about 49: 1: 14, or in other words the yolk occupies a little more than $\frac{3}{4}$ and the protoplasm a little less than $\frac{1}{4}$ of the volume of the entire egg. This relatively large quantity of heavy yolk makes it possible to displace nuclei and cytoplasm in any direction and to study the effects of this on later development (p. 328).

2. While the greater portion of the cytoplasm may be displaced by the yolk a small residual portion of viscid spongioplasm is left between the yolk spheres and in a peripheral layer around the egg; this spongioplasm also forms a framework throughout the entire cell and connects nucleus and centrosphere of resting stages, or mitotic figure of dividing ones, to the peripheral layer.

Because of this framework the stratification of egg substances in centrifuged eggs is never complete, but strands of spongioplasm prevent the free movement and stratification of substances according to their relative weights (pp. 329, 333).

3. The spongioplasm is highly elastic and contractile and when it is stretched or distorted it tends to come back to its normal form and to bring back to their normal positions displaced constituents of the cell (pp. 369, 373).

4. Mitotic figures, especially after the metaphase, are more firmly bound to the peripheral layer than are resting nuclei and centrospheres; the latter are always firmly united and the centrospheres are connected to the peripheral layer of the cell at the point nearest to the animal pole (pp. 333–335, 351–353).

5. As a result of these connections mitotic figures as a whole can be displaced only before the metaphase; after that stage they may be stretched or distorted but their astral radiations can rarely be separated from the peripheral layer (pp. 336, 337, 349).

6. Centrospheres and nuclei of resting stages may be displaced in any direction, but because of their connections with each other and with the periphery they always maintain a definite axial relation, the centrospheres lying between the nuclei and that portion of the periphery which is nearest the animal pole. Nucleus, centrosphere, mitotic figure—each has a polarity of its own, but all are held together in a definite relation to the cell body by the spongioplasm (pp. 352, 362, 363).

7. The persistence of the original polarity in centrifuged eggs in which most of the parts have been displaced and the return of those parts to their normal positions is due to these connections of spongioplasm, which are elastic and contractile (pp. 358, 374).

8. There is no good evidence that the polarity of a cell is a resultant of the electric charges carried on colloidal particles or on cell membranes. When eggs are caused to fuse together each component preserves its own polarity (pp. 366, 368).

9. Neither external nor internal surface tension phenomena are able to explain satisfactorily the persistence of cell polarity in centrifuged eggs (pp. 367, 368).

10. The spongioplasm of the egg of Crepidula is the interalveolar or continuous substance within which are found enchylemma, microsomes, mitochondria, as well as yolk, oil and other inclusions. The form taken by this otherwise continuous substance depends largely upon its relation to these other included substances and it does not consist of preformed fibers or other structures which are definite in number and position. It is most abundant at the animal pole of normal eggs from which it radiates as a spongework between the other inclusions growing more and more coarse as it approaches the vegetal pole (pp. 369–376).

11. Protoplasmic flowing and intracellular movements are probably caused by the contractility of the spongioplasm. It contracts to points of stimulation, such as the entrance point of the spermatozoon, the centrosomes of mitotic figures, etc. Very small inclusions, such as pigment, may be carried along with the spongioplasm in its contraction; larger inclusions such as yolk spheres are forced out of the regions where spongioplasm concentrates (pp. 376–378).

12. The orientations of development such as polarity, symmetry, localization of inclusions, pattern of cleavage, etc. are largely determined by the structure and activities of the spongioplasm, which probably differ in different parts of the egg and at different stages of development (pp. 378–381).

13. The division plane between daughter cells always passes through the equator of the mitotic spindle and at right angles to its axis. If both poles of the spindle are attached to the periphery of the cell it cannot be moved except by very violent centrifuging; if one pole is attached the other may be deflected to one side or the other; if neither pole is firmly attached the entire spindle may be moved. In normal maturation divisions one pole of the spindle is attached to the periphery at the animal pole of the egg and during division this aster is absorbed into the peripheral layer, the spindle grows very short and when the division wall forms through the equator of the spindle it cuts off a minute polar body from a relatively enormous egg (p. 336).

14. If yolk is forced to the animal pole after the maturation spindle has become attached to the periphery the spindle is stretched in length and when the division plane forms through its equator it cuts off a giant polar body or may divide the egg equally. Giant polar bodies do not develop because they do not receive a spermatozoon, and they do not receive a spermatozoon because they are formed after the fertilization of the egg and after the entire cortical layer has been rendered impervious to the entrance of other spermatozoa(pp. 336–344).

15. If yolk is forced to the animal pole before the maturation spindle has become attached to the periphery the spindle may be driven to any point on the egg surface and if held there by continued centrifuging either one or both polar bodies may be formed there. Nevertheless nuclei and cytoplasm move back to the animal pole and yolk to the vegetal pole when centrifuging ceases and the polarity of the egg and embryo remains unchanged. Therefore the maturation pole does not determine the animal pole of the egg nor the ectodermal pole of the embryo (pp. 335, 336, 346, 347).

16. By centrifuging during cleavage all the yolk may be driven into one daughter cell and most of the cytoplasm into the other one, or by centrifuging early in mitosis the spindle may be carried out of its normal position so that the first or second cleavage may be equatorial instead of meridional, unequal instead of equal. Nevertheless the cells formed by the first two cleavages behave like normal macromeres in that each gives rise to three micromeres (ectomeres) on its animal pole side in the three succeeding cleavages (pp. 349–354).

17. If the first or second sets of micromeres are forced to form at a distance from the animal pole the succeeding set forms at the animal pole if the pressure is removed. If the unequal cleavages by which micromeres are formed normally are rendered equal by centrifuging the subdivisions of these large "micromeres" are normal only so far as the cells are concerned which lie nearest the animal pole. This is due to the fact that under normal conditions the upper pole of a spindle is attached to the periphery at the animal pole side of the cell more firmly than is the lower pole of the spindle (pp. 354–356).

18. The differentiation of daughter cells does not depend upon a differentiation of their centrosomes or nuclei, for the spindles may be turned about without changing the differentiation; nor does it depend upon the segregation of the movable parts of the cytoplasm or of the yolk in one cell or the other, for these segregations may be reversed without changing the differentiations; nor does it depend entirely upon the position and direction of the mitotic figure and the cleavage plane with reference to the egg axes, for these may be forcibly changed as in equatorial first or second cleavages without changing the normal course of differentiation in those cells after the force has ceased to act. These may be contributory factors in the differentiation of cells, but the principal factor is evidently to be found in the spongioplasm which always tends to come back to its normal form if it is stretched or distorted, and which probably differs in structure in different parts of the egg and in different stages of development (pp. 357–360).

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DESCRIPTION OF FIGURES

All figures represent entire eggs of Crepidula plana, fixed, stained, and mounted on slides in balsam. They were drawn by means of a camera lucida with Zeiss apochromatic oil immersion Obj. 3 mm., Ocular 4, at stage level and are therefore magnified 333 diameters. In the process of reproduction they have been reduced one-third.

The oil droplets, indicated by a coarse aveolar structure, mark the centripetal pole; the great mass of yolk, which is left unshaded, lies in the centrifugal half of the egg; while the cytoplasm of the middle zone is shaded by stipples, the more granular part (spongioplasm) being stippled more densely than the less granular part (hyaloplasm) when these two are separated. The eggs drawn usually represent common types of abnormalities produced by centrifuging. Thousands of other kinds of abnormalities are produced, indeed no two are ever identically the same.

PLATE 1

EXPLANATION OF FIGURES

1 and 2 (1126, 1) Eggs taken while being laid and centrifuged in the germinal vesicle stage for 10 minutes; fixed immediately after centrifuging. The segregation of yolk and cytoplasm is fairly complete. The eggs are flattened in the axis of centrifuging and the nuclei are elongated in that axis; the chromosomes and centrosomes are at the heavier end of the nucleus, the nucleolus is at the lighter end. The grouping of the chromosomes shows that the axis of the future spindle will be oblique to the long axis of the nucleus in figure 1, transverse to it in figure 2.

3 and 4 (1125, 1) Centrifuged 10 minutes (3000 revolutions per minute), fixed at once. The nucleolus is thrown with the fatty substance to the lighter pole of the egg; the sperm nucleus remains in the yolk in figure 3, in the cytoplasm in figure 4. The two poles of the spindle are equidistant from the cell surface and are not in contact with it. Figures 3, 4, 7, 8, are from the same slide, figures 9 to 12 are from the same experiment 1½ hours after centrifuging. Eggs of this same lot 24 hours after centrifuging show both polar bodies in the middle of the ectodermal plate and everything absolutely normal.

5 and 6 (1038) Centrifuged 10 minutes, fixed 3 hours later. The cytoplasm has been moved to the vegetative pole. The first maturation spindle has remained attached to the animal pole and its traction on the cell membrane is shown by the indentation of the latter. The sperm nucleus lies near the fatty substance of the lighter pole of the egg in figure 5, and in the yolk in figure 6. In figures 5 and 6 the spindle was attached to the cell membrane at the animal pole before centrifuging began.

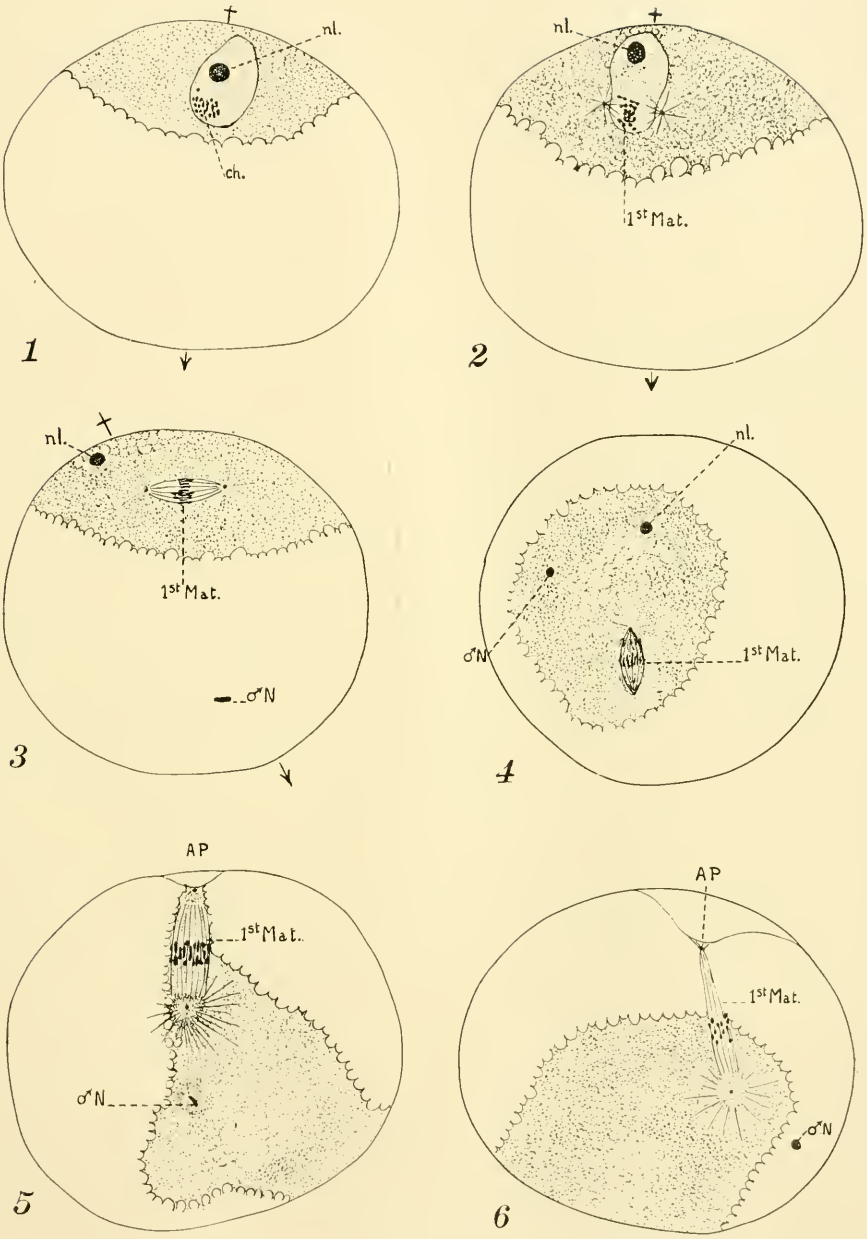
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PLATE 2

EXPLANATION OF FIGURES

7 and 8 (1125, 1) Centrifuged 10 minutes (3000 revolutions per minute); fixed at once. Anaphase of first maturation division; the spindle has been somewhat elongated and in figure 8 the central end of the spindle has been thrown out into a lobe containing oil drops; the sperm nucleus lies in the yolk.

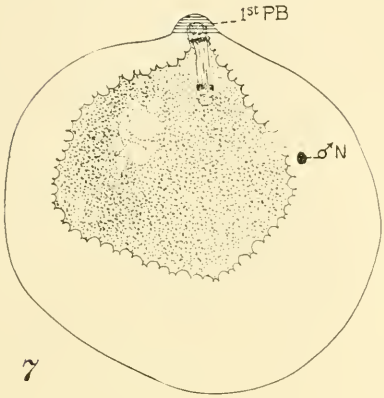
9 to 12 are all from the same slide. The eggs were centrifuged for 10 minutes, in the prophase of the first maturation division and were fixed 1½ hours later. The spindles were moved from the animal pole and during the hour and a half which elasped after centrifuging the spindles advanced to the anaphase, but made no progress toward the present protoplasmic pole of the egg. On the other hand there are evidences that they are progressing slowly toward the original animal pole which is now occupied by yolk. In subsequent stages eggs of this lot show both polar bodies at the ectodermal pole and almost all eggs have developed normally. This seems to indicate that in this lot of eggs the first maturation spindle moves back to the original animal pole and that the polar bodies are formed there.

9 (1125, 1) First maturation spindle near the middle of the egg, on the boundary between yolk and cytoplasm. Sperm nucleus in cytoplasm near surface.

10 (1125, 2) Late anaphase of the first maturation mitosis. The spindle lies in the middle of the egg with neither pole near the cell membrane; there is no trace of cell constriction. The spindle is apparently moving from the cytoplasm into the yolk.

11 (1125, 2) Late anaphase of first maturation mitosis; the spindle lies in the middle of the egg, almost surrounded by yolk.

12 (1125, 2) Anaphase of the first maturation mitosis. The spindle lies on the boundary between the yolk and the cytoplasm and is somewhat bent and distorted.



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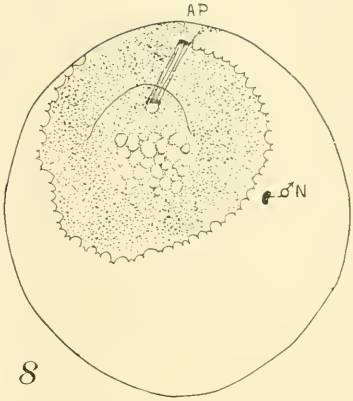

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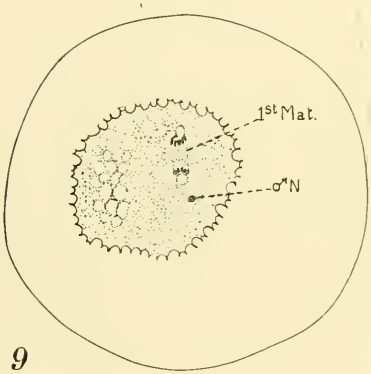

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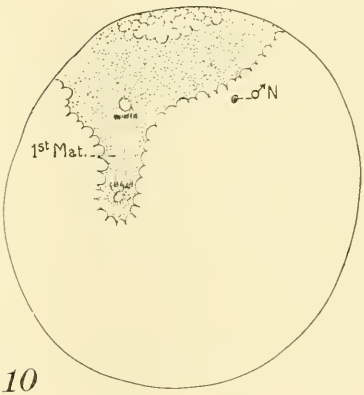

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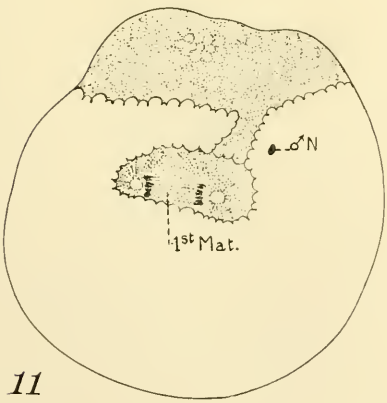

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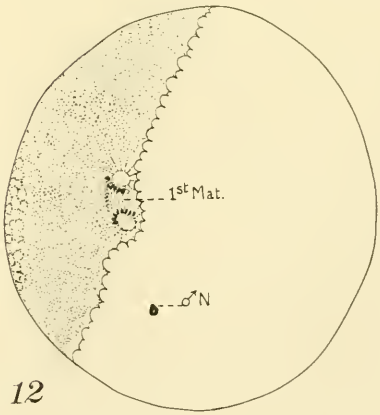

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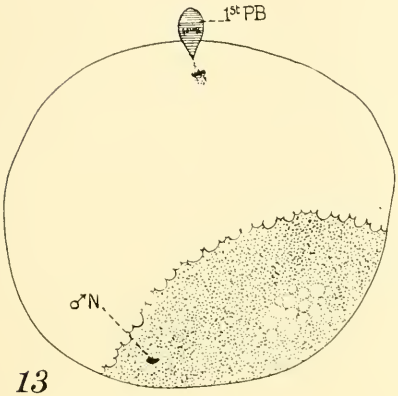
PLATE 3

EXPLANATION OF FIGURES

13 (1125, 1) Centrifuged 10 minutes, fixed at once. The first polar body was formed, before centrifuging, at the original animal pole; this was the centrifugal pole and consequently the yolk was thrown to this pole while the cytoplasm was forced to the vegetal pole; the half of the first maturation spindle left in the egg remains attached to the animal pole by the spindle fibers.

14 to 18 All from same slide (1125, 2); centrifuged 10 minutes, fixed 1½ hours after. Centripetal pole marked by oil droplets. At time of centrifuging the first maturation spindle was attached to the surface of the egg at the animal pole and therefore was not moved; consequently the first polar body has formed at that pole. The cytoplasm was displaced more or less from the animal pole but is now returning, as is shown by figures 15 and 16 in which the centripetal pole (marked by the oil droplets) does not lie in the middle of the cytoplasmic field, and by figures 17 and 18 in which a narrow lane of cytoplasm is returning to the animal pole (cf. figure 13 in which the eggs of this experiment were fixed at once after centrifuging).



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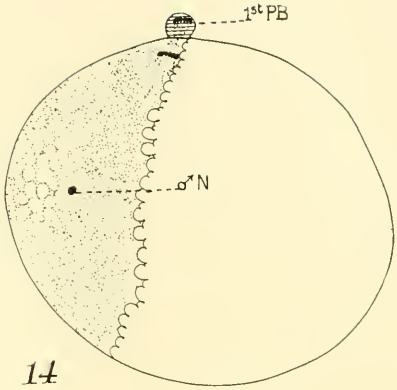

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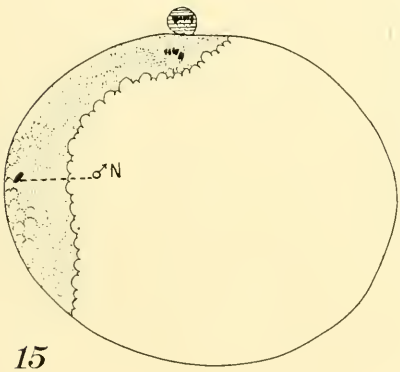

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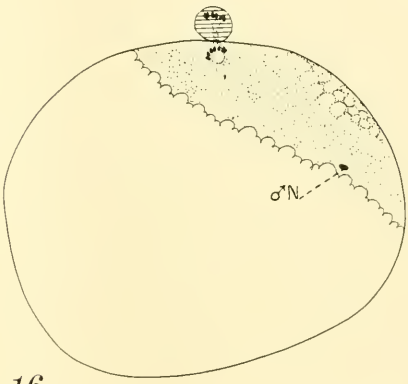

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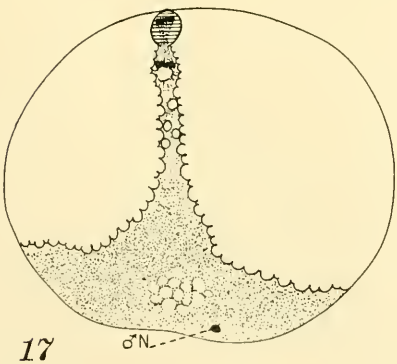

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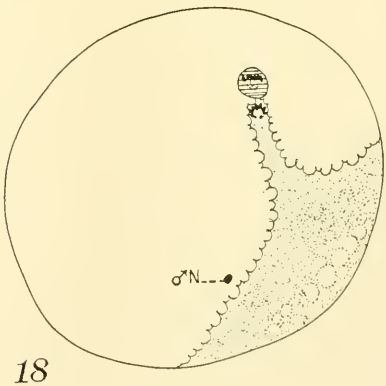

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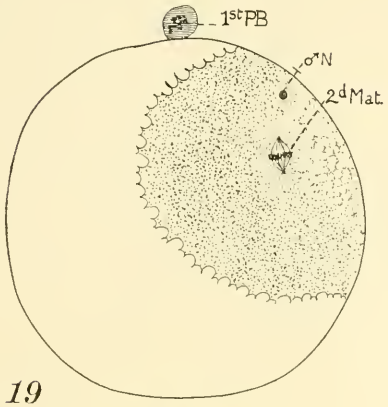
PLATE 4

EXPLANATION OF FIGURES

19 to 21 (1037) Centrifuged 10 minutes in the prophase of the second maturation division; fixed at once. The spindle was not firmly attached to the surface layer and was carried with the cytoplasm to the centripetal pole.

22 (1088) Centrifuged 100 turns of hand machine in about 1 minute; fixed 1 hour later. The second maturation spindle in the anaphase remains anchored to the cell membrane at the animal pole, but is stretched in length by the centrifuging. The sperm nucleus lines near the animal pole.

23 to 27 (1038) Centrifuged 10 minutes; fixed 3 hours later. The cytoplasm has been displaced from the animal pole; the second maturation spindle remains attached to the surface at that pole, but is greatly stretched in length. In figures 23 to 25 the cell constriction shows a giant polar body in the process of forming; only the cell containing the ♂ N will develop, in figure 23 the portion nearest the animal pole, in figure 24 that nearest the vegetal pole; in figures 26 and 27 a giant second polar body has been cut off. All nuclei and centrospheres within the yolk are much smaller than those within the cytoplasm.



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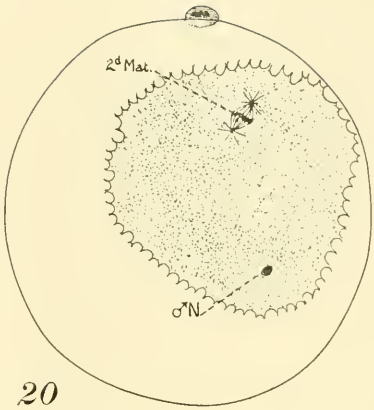

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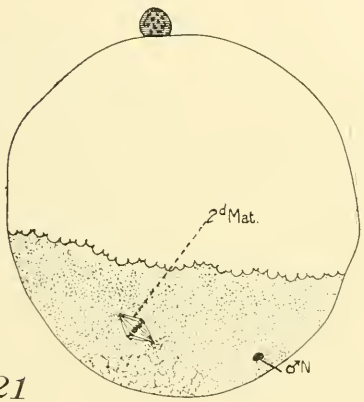

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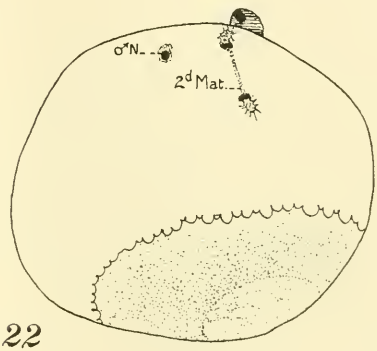

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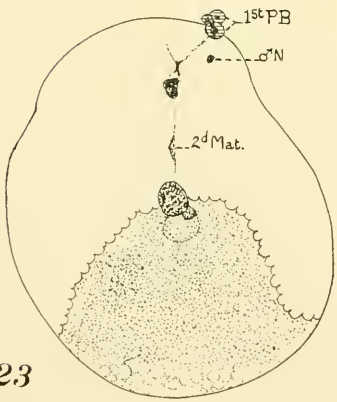

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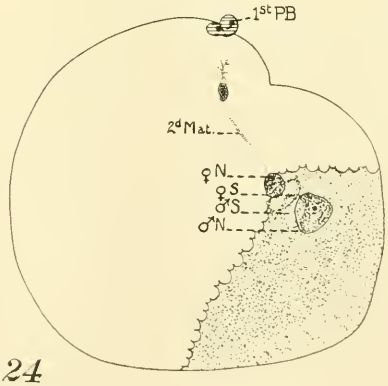

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PLATE 5

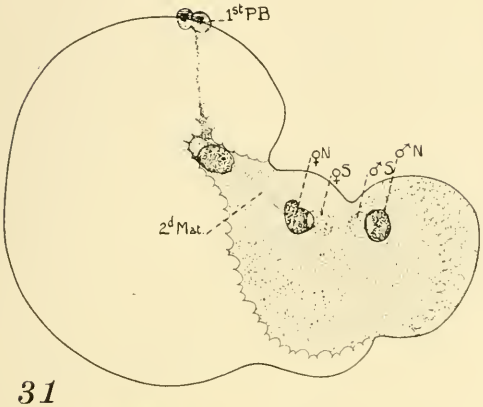
EXPLANATION OF FIGURES

Figures 25–27 are explained on p. 394.

28 Normal egg showing the usual distribution of cytoplasm and yolk and the usual relative sizes of egg and sperm nuclei and spheres.

29 (1088) Centrifuged 100 turns of hand machine; fixed 1 hour later. Cytoplasm lies at vegetal pole, and the sperm nucleus which lies in it is much larger than the egg nucleus.

30 (1125, 2) Centrifuged 10 minutes; fixed 1½ hours later. The sperm nucleus is immense owing to its position in the cytoplasm, the egg nucleus is small owing to its position in the yolk.



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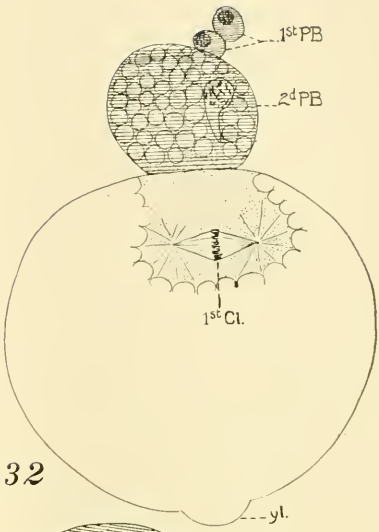

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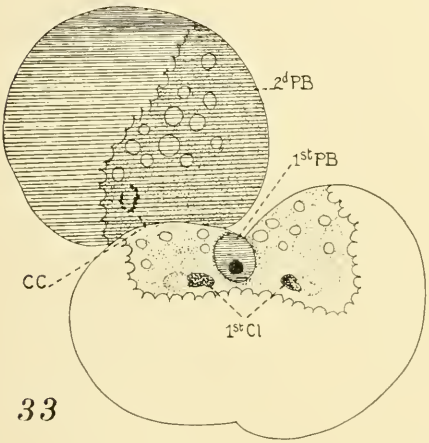

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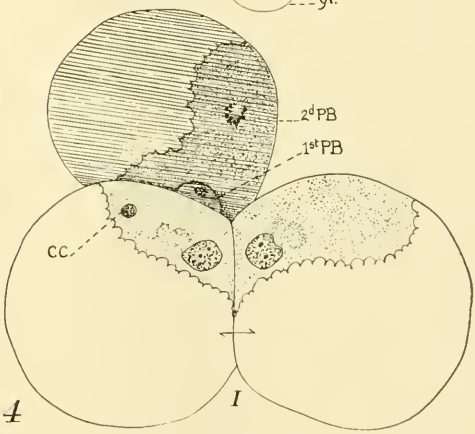

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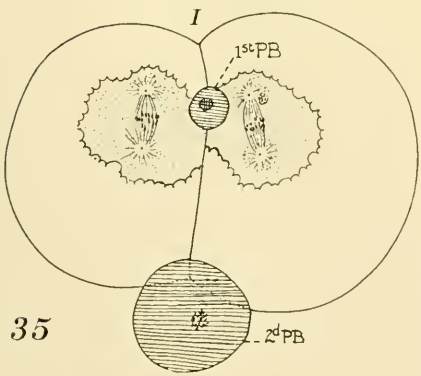

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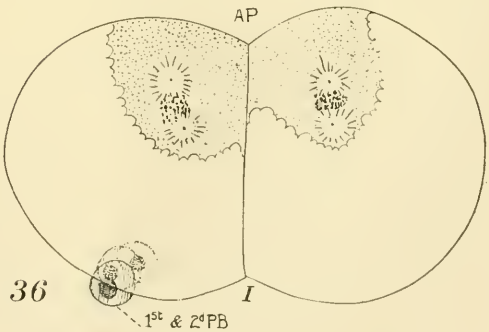

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PLATE 7

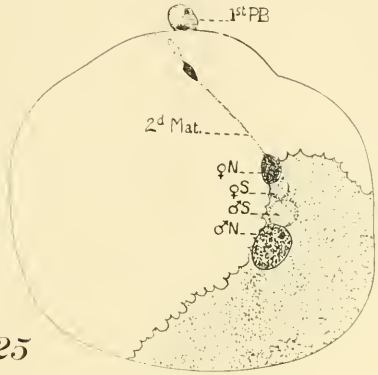
EXPLANATION OF FIGURES

37 to 40 (1146) The polar bodies are large and protoplasmic and contain oil droplets (first polar body has dropped off in figures 39 and 40), showing that they were extruded at the centripetal pole; after centrifuging ceased the cytoplasm and nuclei moved back to the present protoplasmic pole.

41 (1145) The first polar body was formed normally before centrifuging began; the second was extruded during centrifuging and is much larger than normal and lies at a distance from the first. Cytoplasm and nuclei moved back to the true animal pole after centrifuging and the egg continued to develop normally.

42 to 54 (1136) Centrifuged 30 minutes; fixed from 6 to 24 hours later. In some cases the first polar body is normal and lies at or near the animal pole (figs. 42, 47, 54) showing that it was formed before centrifuging; in all cases the second polar body is much larger than normal and lies at some distance from the animal pole, showing that it was extruded during centrifuging; and in all cases cytoplasm and nuclei returned more or less completely to the animal pole after centrifuging ceased and the further development is normal in most cases. Chromosomes of a second polar body rarely give rise to a resting nucleus, showing that a centrosome is necessary for this process.

42 First polar body is normal and lies near animal pole; second is very large and contains cytoplasm and oil, showing it was formed at centripetal pole.



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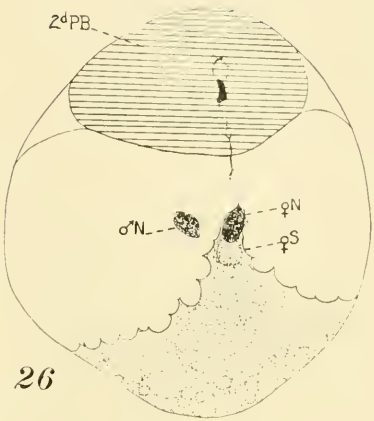

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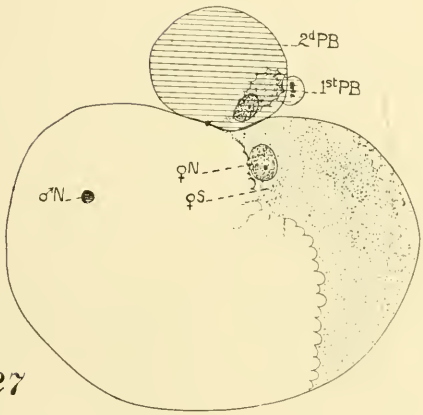

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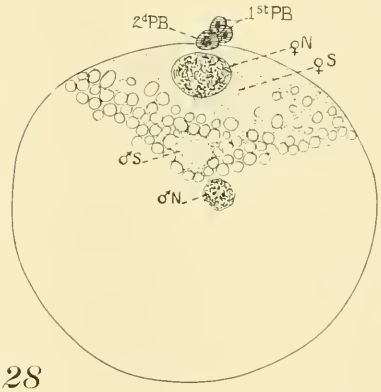

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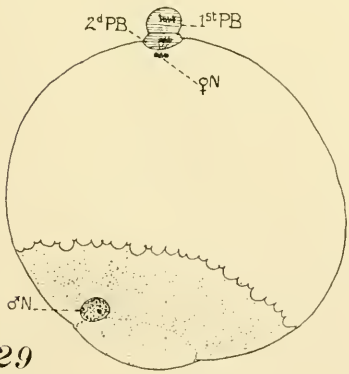

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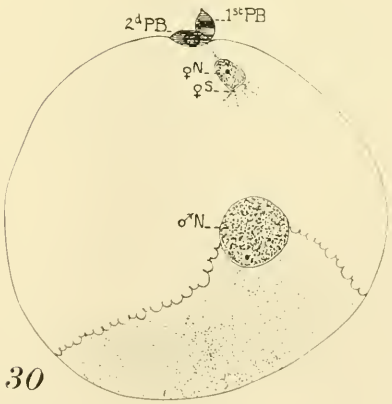

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PLATE 6

EXPLANATION OF FIGURES

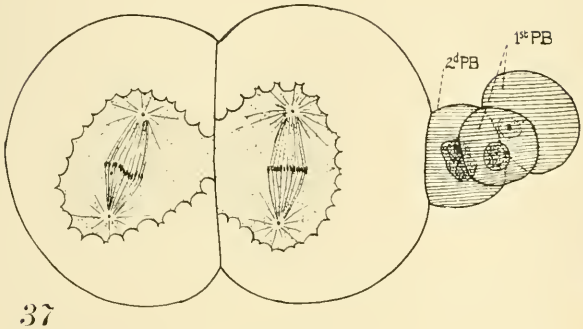
31 (1038) Centrifuged 10 minutes; fixed 3 hours later. Normal first polar body has divided. Giant second polar body forming, as indicated by constriction opposite middle of second maturation spindle; latter connected to animal pole. Sperm nucleus and sphere in cytoplasmic lobe on right; adjoining this the egg nucleus and sphere.

32 to 34 (1040) Centrifuged 10 minutes; fixed 5 hours later. Figure 32. Normal first polar body has divided; giant second polar body contains spherules and peculiar (telophase) nucleus; first cleavage spindle (metaphase) in egg. Figures 33 and 34. Telophase of first cleavage. First polar body normal; giant second polar body containing cytoplasm and yolk, chromosomes have not formed a resting nucleus; *cc* scattered chromaten near mid-body of second maturation spindle.

35 to 41 (1145, 1146) Centrifuged 4 hours (2000 revolutions per minute); fixed 6 hours later. All eggs are in the second cleavage stage and most of them continued to develop normally. Most of the eggs figured (figs. 33, 34, 35, 41) were centrifuged after the extrusion of the first polar body at the animal pole, but before the formation of the second polar body. The latter is a giant polar body and is usually extruded at some distance from the animal pole. In figures 36, 37, 38, and possibly figures 39 and 40 also, the eggs were centrifuged during the formation of both polar bodies, and consequently both are displaced from the animal pole. In these eggs the maturation pole was forced away from the animal pole, but the cytoplasm and the nuclei went back to the latter when centrifuging ceased.

35 (1145) The smaller (first) polar body evidently marks the animal pole, the second was formed some distance from the pole.

36 (1146) Polar bodies larger than normal, but they contain no yolk. The maturation spindles together with the cytoplasm were evidently forced away from the animal pole, and the polar bodies formed at this centripetal pole; afterwards the cytoplasm and nuclei moved back to their present position. Spindles present for the second cleavage.



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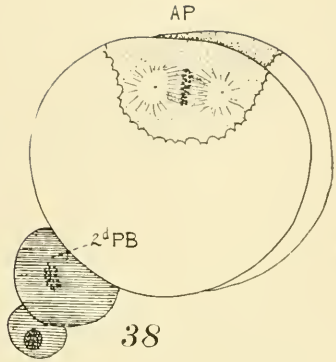

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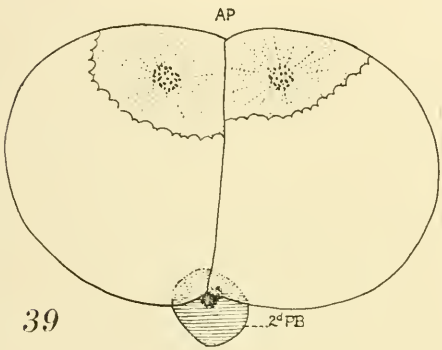

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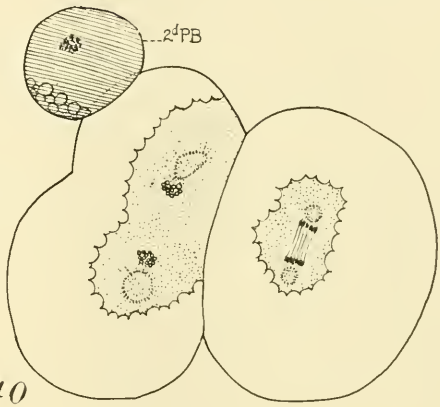

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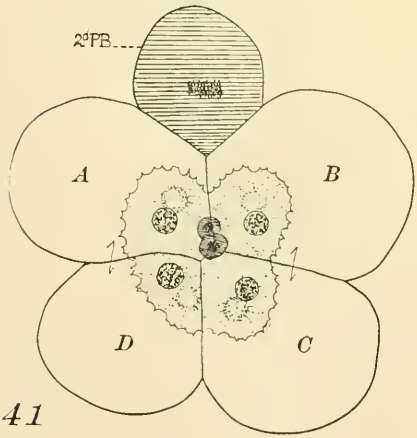

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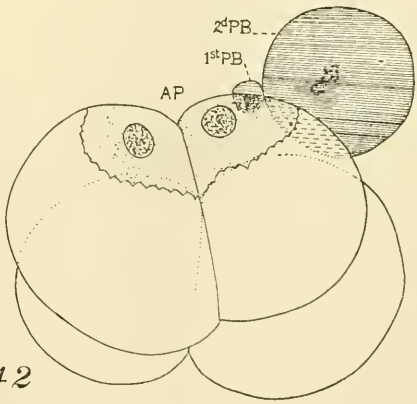

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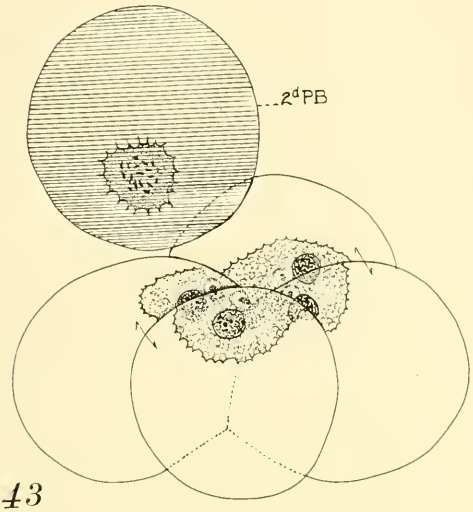
PLATE 8

EXPLANATION OF FIGURES

43 to 46 First polar body has dropped off. Enormous second polar body, containing a large amount of yolk, lying near animal pole in figures 43 and 45, at vegetal pole in figures 44 and 46; evidently formed as shown in figures 23 to 27. Only that portion of egg develops which contains a spermatozoon, and any portion whether at animal or vegetable pole which contains a spermatozoon may develop.



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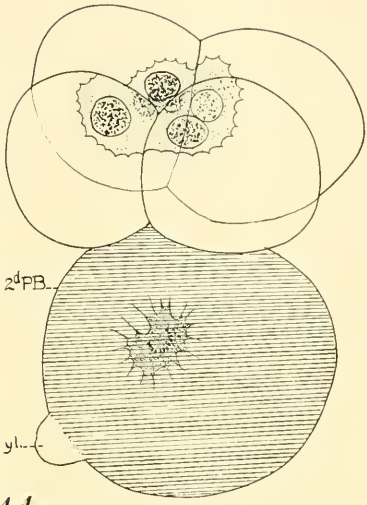

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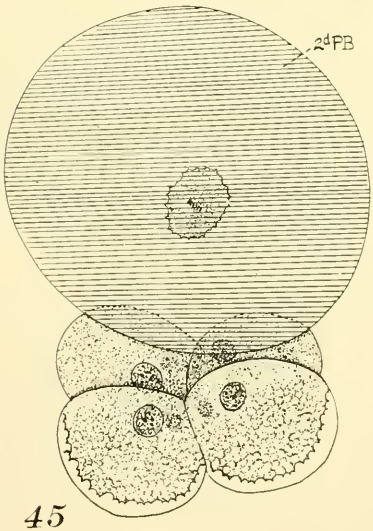

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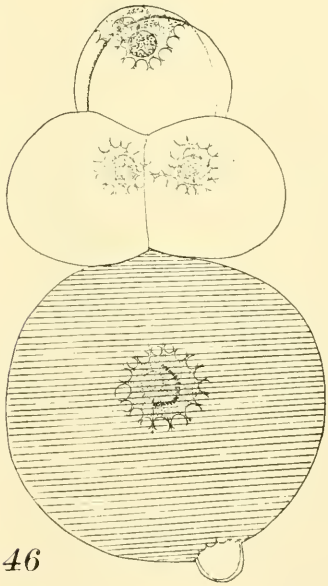

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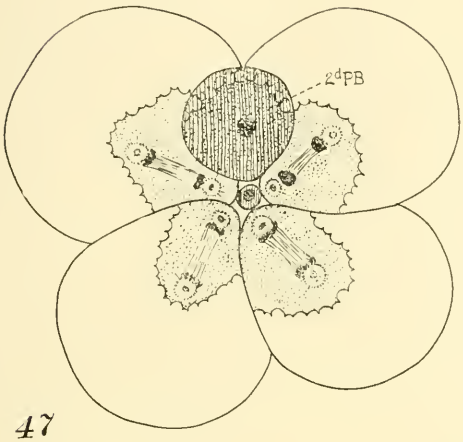
PLATE 9

EXPLANATION OF FIGURES

47 Normal first polar body at animal pole; giant second polar body a little to one side of the animal pole; it contains cytoplasm and oil and was therefore formed at the centripetal pole.

48 Original animal pole probably indicated by the small first polar body; the large second polar body was formed some distance from this. During the first, second and third cleavages the original polarity was not completely restored but the positions of the micromeres and of their nuclei and centrospheres indicate that there has been an attempt to restore the original polarity; in the upper, yolk-laden micromeres, cytoplasm, nuclei and centrospheres are turned away from the animal pole and toward those of the lower micromeres. Therefore the micromere pole is somewhat removed from the original animal pole.

49 to 54 The giant first and second polar bodies do not lie at the middle of the plate of micromeres (ectodermal pole), though the normal first polar body in figure 54 does; the ectodermal pole therefore coincides with the original animal pole. All the micromeres are quite normal in number, size and position.



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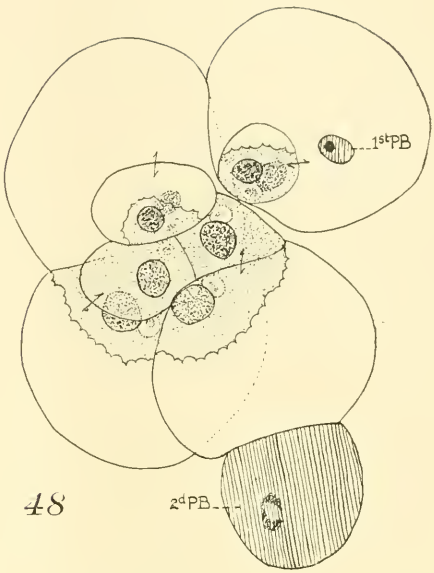

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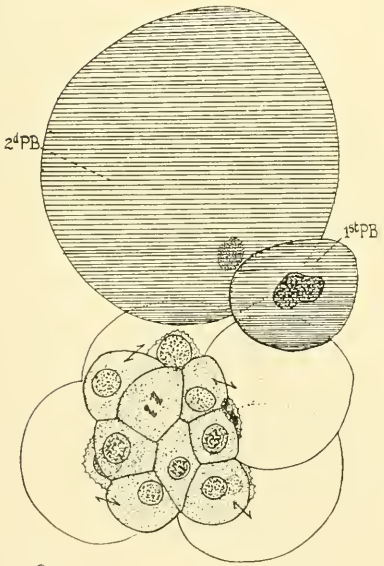

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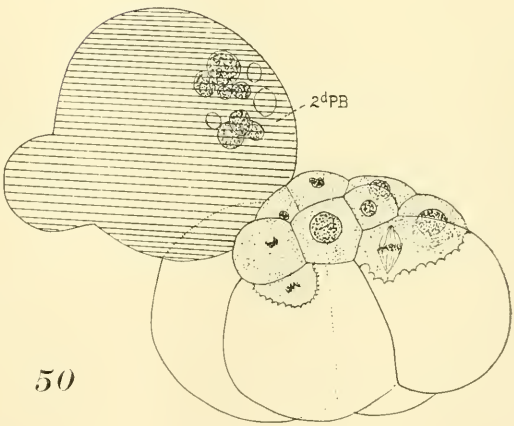

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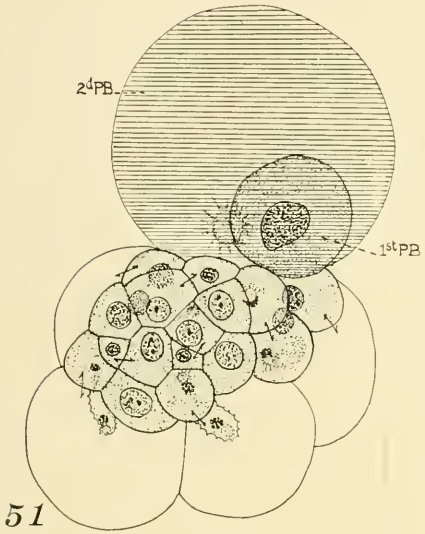
PLATE 10

For explanation of figures 51–54 see page 404



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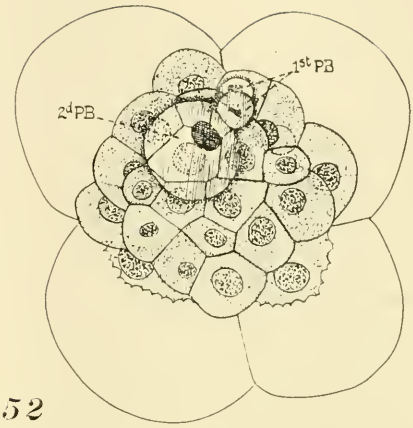

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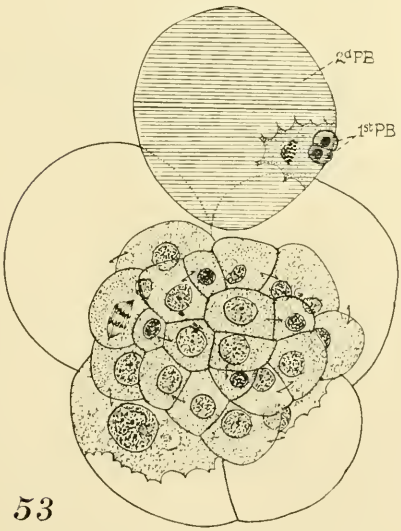

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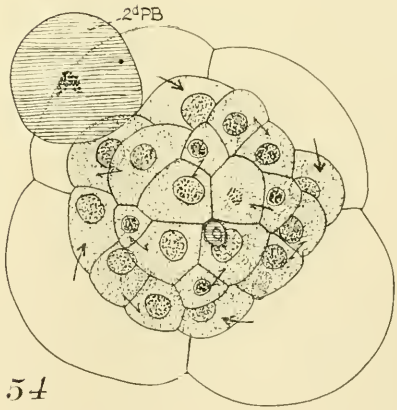

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PLATE 11

EXPLANATION OF FIGURES

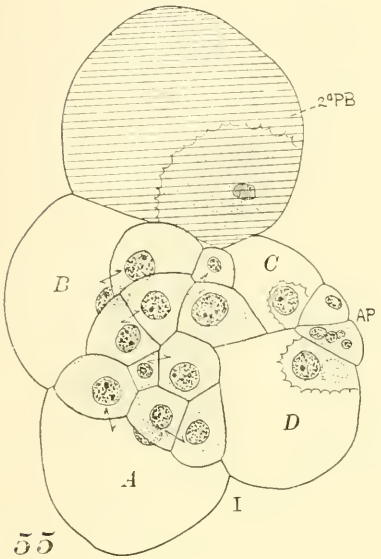
55–58 Eggs in which the original polarity has been more or less modified.

55 (1147) Centrifuged 4 hours, fixed 18 hours later. This figure may be interpreted as follows:—The original animal pole was probably at the point *AP* (the first polar body has been lost). The cytoplasm and second polar spindle were carried away from this pole and a giant second polar body was formed near the equator of the egg. Cytoplasm and nuclei did not return to the animal pole before the first cleavage (*I*) which was consequently equatorial, as in figure 46. The micromeres formed from *A* and *B* are normal, though removed from the original animal pole; those from *C* and *D* are abnormal though lying at that pole.

56 (1136, 5) Centrifuged 30 minutes, fixed 7 hours later. The first polar body marks the position of the original animal pole, at which point two micromeres now center. The second polar body was formed at the vegetal pole as indicated by the presence on it of the yolk lobe (*yl*). At the first or second cleavage the macromeres were partially separated and turned so that the poles at which the micromeres form do not coincide in each quadrant except in quadrants *A* and *B*. Each macromere has formed one micromere which is normal in size and appearance in quadrants *A* and *C* but which contains yolk in quadrants *B* and *D*.

57 (1138) Centrifuged 30 minutes, fixed 12 hours later. At the upper side of the figure is a large lobulated second polar body containing two masses of chromatin and three centrospheres; it contains oil and cytoplasm and was extruded at the centripetal pole. The two lower macromeres are partially separated from the other two and contain but little cytoplasm and much yolk. Each macromere has given off two micromeres which are abnormal in contents and position in the lower quadrants but entirely normal in the upper ones, where the micromeres of the first set are dividing.

58 (1136, 4) Centrifuged 30 minutes, fixed 12 hours later. The first polar body had formed at the animal pole before centrifuging; a giant second polar body was then extruded at the vegetal pole. Afterward most of the cytoplasm returned to the animal pole, but a portion remained at the vegetal pole, probably cut off by an equatorial first cleavage as in figures 65 to 73.



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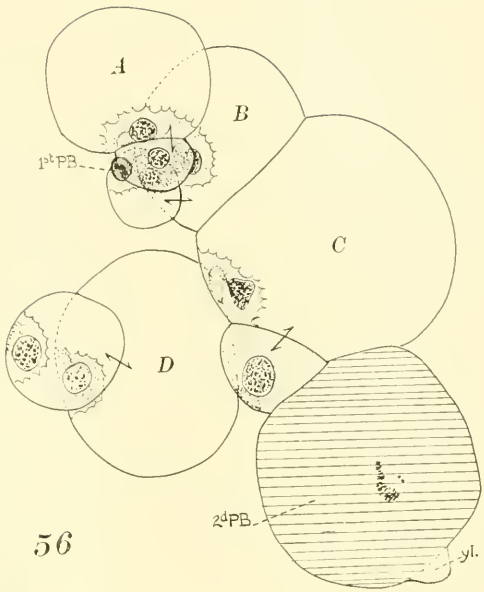

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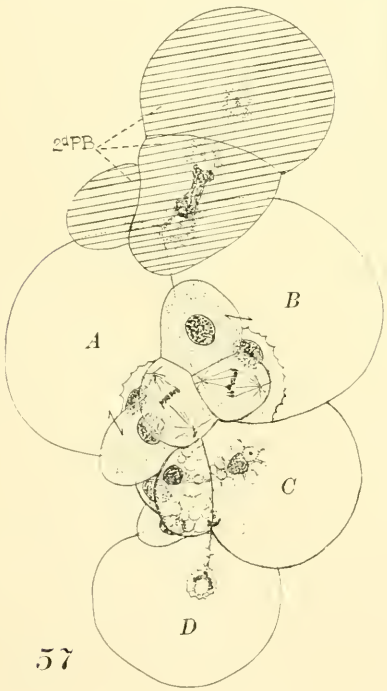

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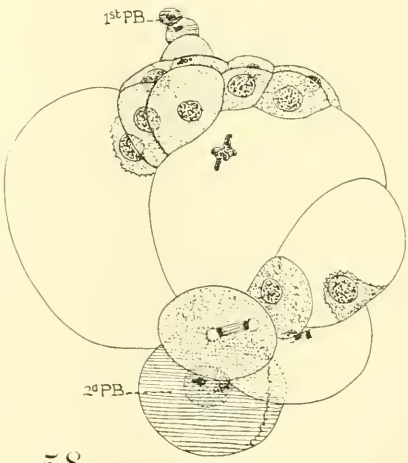


58

PLATE 12

EXPLANATION OF FIGURES

59 to 82 Centrifuged after both maturation divisions but before the completion of the first cleavage.

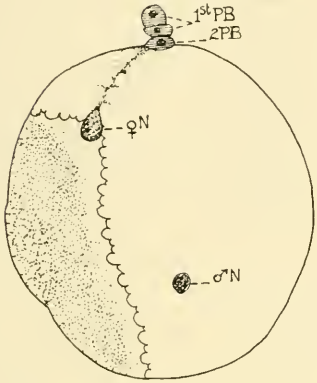
59 (1088) Centrifuged 100 turns of hand machine, fixed 1 hour later. Side view of an egg in which the axis of centrifuging was at right angles to the polar axis. Egg nucleus connected by strands of spongioplasm with the animal pole.

60 (1037) Centrifuged 10 minutes, fixed at once. Yolk was thrown to the animal pole, oil and cytoplasm to the vegetal pole. Both germ nuclei are stretched out in the direction of centrifuging and both have been pulled away from the animal pole. The egg nucleus is still connected with that pole by strands of granular cytoplasm (spongioplasm) and there is an aggregation of this immediately under the polar bodies; other strands run through the zone of spongioplasm and of liquid yolk into the zone of yolk spherules.

61 (1037) Centrifuged 10 minutes, fixed at once. The germ nuclei and cytoplasm are displaced somewhat from the animal pole and the nuclei are distorted.

62 (1139, 1) Centrifuged $2\frac{1}{2}$ hours, fixed at once. Both germ nuclei together with the cytoplasm have been forced away from the animal pole.

63 to 65 (1037) Centrifuged 10 minutes, fixed at once. The first cleavage spindle in the anaphase has been forced away from the animal pole, the spindle is more or less distorted and the centrosomes and spheres are turned toward the animal pole. The lobes (*L*) containing oil at the centripetal pole indicate that the centrifugal force was strong.



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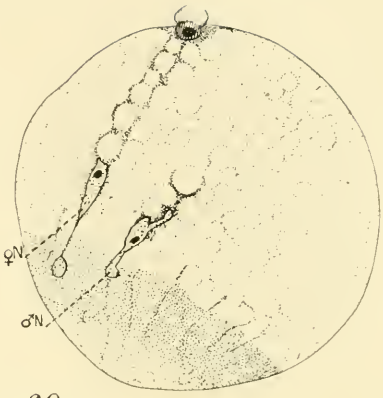

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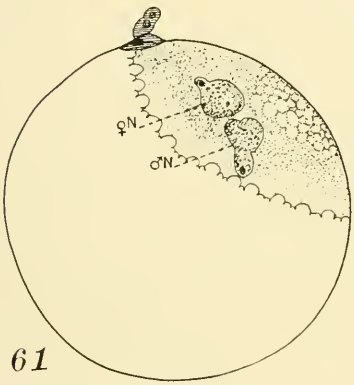

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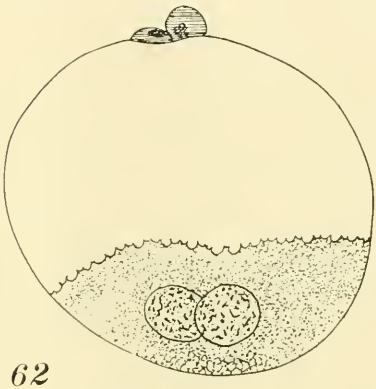

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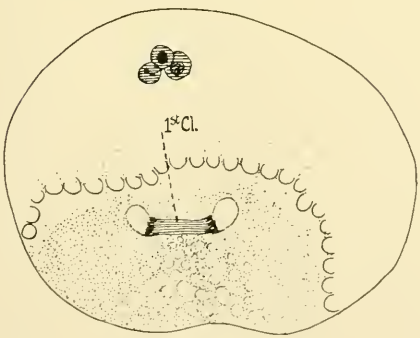

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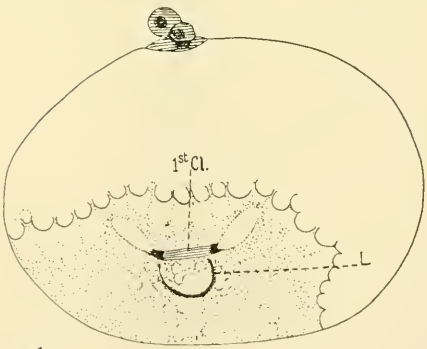


64

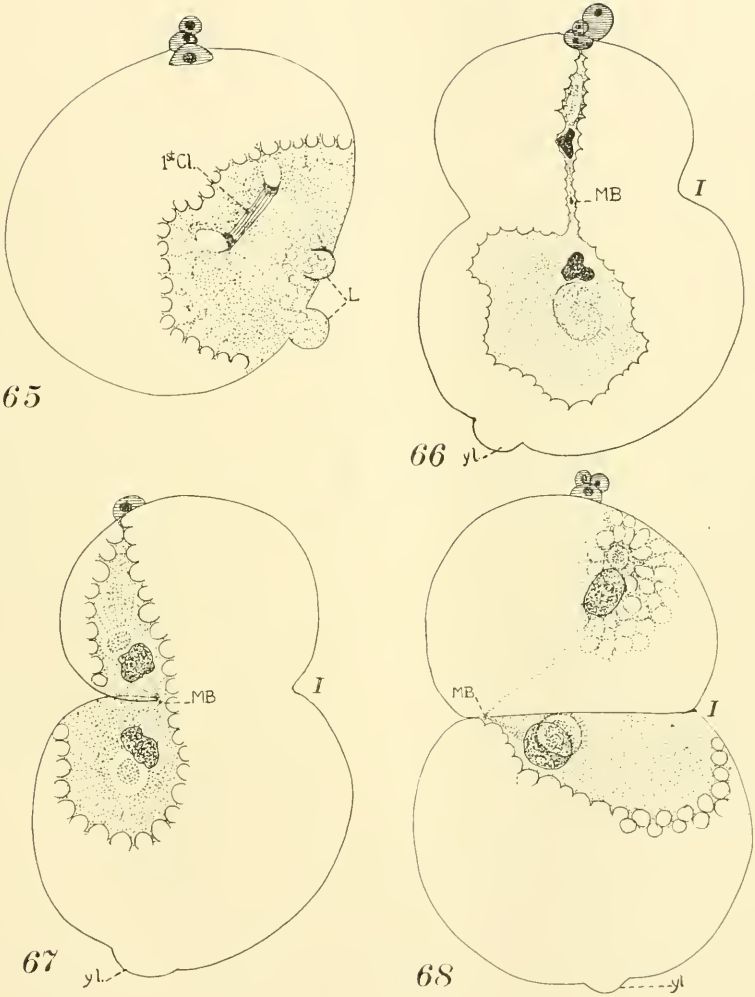
PLATE 13

EXPLANATION OF FIGURES

Figure 65 is described on p. 410.

66 (1040) Centrifuged 10 minutes, fixed 5 hours later. The first cleavage spindle has been turned into the polar axis and the cleavage furrow is appearing in an equatorial position. The animal pole is marked by the polar bodies, the vegetal pole by the yolk lobe (*yl*). Development delayed about three hours.

67 to 70, 72 (1035) Centrifuged 30 minutes, fixed 3 hours later. First cleavage equatorial. After centrifuging, the cytoplasm, nuclei and centrospheres in the lower cells move as near as possible to the animal pole. One end of the cleavage spindle remains near the animal pole, to which it is probably attached. In figure 70 the centrosome which remained near the animal pole was separated from its nucleus and has divided twice; the chromosomes and a portion of the cytoplasm in the upper cell lie near the equator adjoining the cytoplasm and spindle in the lower cell; the polarity is modified to this extent. In figure 72 the second cleavage is beginning in a meridional axis and nearly at right angles to the first (equatorial) cleavage (1).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L
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PLATE 14

EXPLANATION OF FIGURES

For descriptions of figures 69, 70 and 72 see page 412.

71 (1146) Centrifuged 4 hours, fixed 6 hours later. The first cleavage (*I*) was nearly equatorial; the second cleavage spindle is at right angles to the first in the upper cell, and nearly parallel with the first in the lower cell.

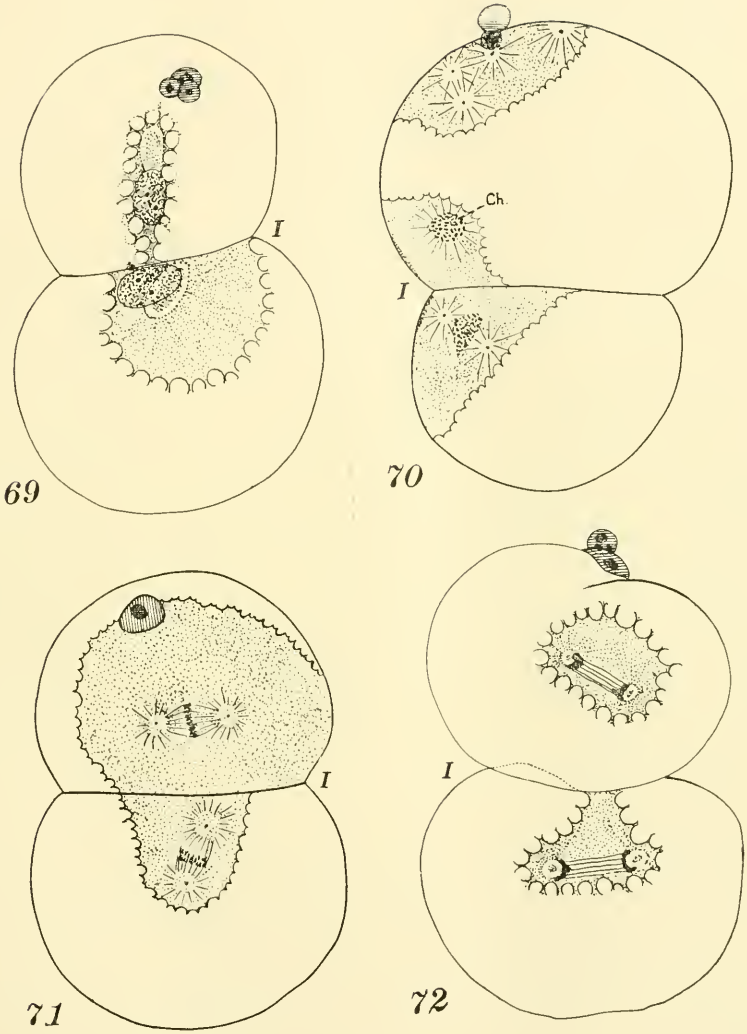

I
69
Ch.
I
70
I
71
I
72

PLATE 15

EXPLANATION OF FIGURES

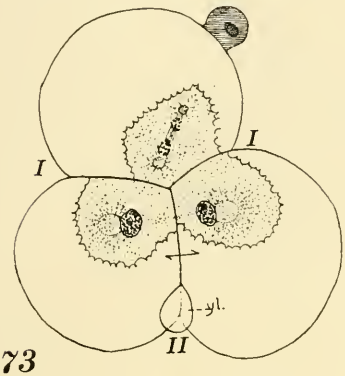
73 (1089) Centrifuged 100 turns of hand machine, fixed 4 and 6 hours later. First cleavage (*I*) equatorial; second meridional in cell below equator, oblique and belated in cell above the equator.

74 (1139, 1) Centrifuged 2½ hours at the close of the first cleavage, fixed at once. In the right half of the egg, cytoplasm and nuclear spindle were displaced toward the vegetal pole and were held in that position during the second cleavage; therefore the second cleavage furrow in the right half is nearly equatorial in position and in both daughter cells the centrospheres lie on the animal pole side of the nucleus. In the left half of the egg division is nearly normal, though somewhat belated.

75 (1136, 5) First polar body missing. The second polar body (with two nuclei) contains oil and cytoplasm and was therefore extruded at centripetal pole, which was near vegetal pole of egg. Cytoplasm and nuclei did not return to animal pole before the first cleavage (*I–I*) which was equatorial, consequently cytoplasm and nuclei were cut off in cells below the equator. Positions of cytoplasm, nuclei and spheres show that the animal pole (*AP*) is at top of figure. The position of the cytoplasmic areas in the different cells, as well as that of the midbody and the spindle remnants, prove that furrow running from the top to the bottom of the figure is the second cleavage but the spiral direction of this cleavage is dexiotropic, as shown in the position of the cells and by the polar furrows, whereas in normal eggs this cleavage is laeotropic.

76, 77 (1035) Centrifuged 30 minutes, fixed 3 hours later. The first cleavage (*I*) was equatorial, the second (*II*) meridional. Each macromere is giving off in a dexiotropic direction and as near as possible to the animal pole, a micromere of the first set.

78 (1151) Centrifuged 5 hours, fixed 5 hours later. The first cleavage was equatorial, the second meridional. Each macromere has formed a micromere of the first set and the two macromeres below the equator have also formed the second set of micromeres (*2c, 2d*); the macromeres above the equator are in process of giving off the second set of micromeres.



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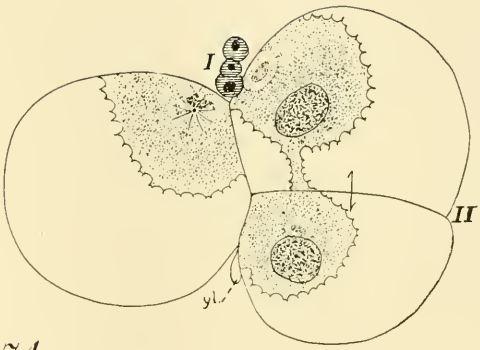

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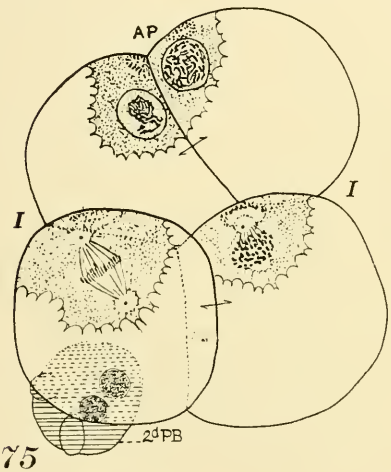

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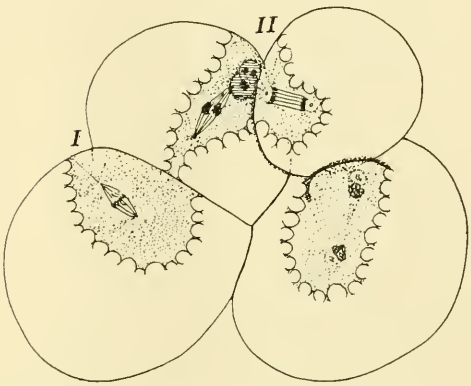

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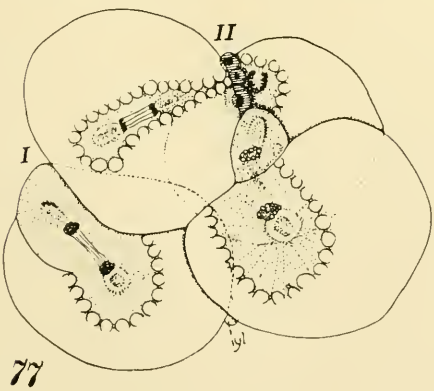

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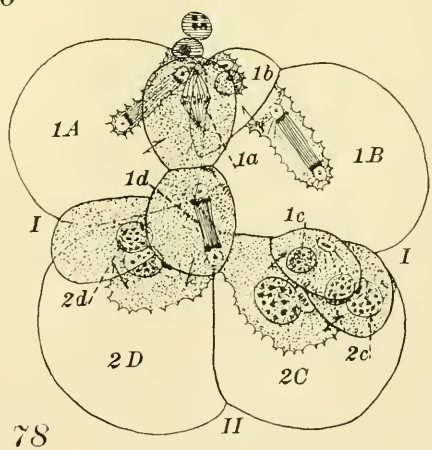

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PLATE 16

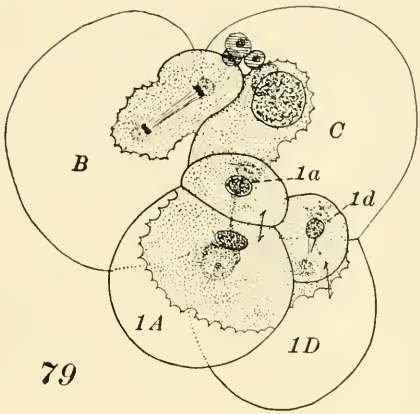
EXPLANATION OF FIGURES

79 (1145) Centrifuged 4 hours, fixed 6 hours later. Cytoplasm and nuclei were displaced toward the vegetal pole and the first cleavage was nearly equatorial, while the second cleavage was meridional. In the four macromeres thus formed the original polarity is preserved as far as possible and micromeres are being formed on the sides of the cells toward the polar bodies.

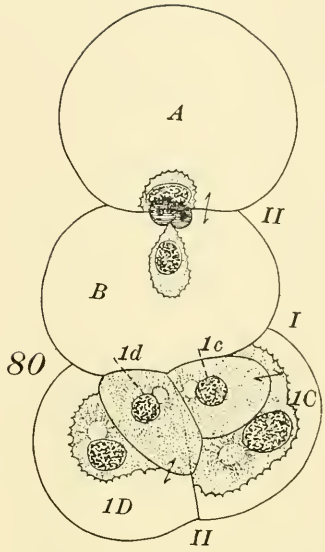
80 (1136, 5) Centrifuged 30 minutes, fixed 7 hours later. The first cleavage (*I*) was oblique as in figure 65; the second was meridional, being at right angles to the first in the lower cell and parallel with it in the upper one. The lower cells contain most of the cytoplasm and give rise to the micromeres of the first set which are reversed in position; the upper cells which contain little cytoplasm have not yet formed micromeres.

81 (1032A) Centrifuged $2\frac{1}{2}$ hours, fixed 21 hours later. Cytoplasm and nuclei were displaced toward the vegetal pole, the first cleavage was nearly equatorial, and the second nearly meridional. Each of these macromeres has formed a micromere of the first quartet around a point, about 90° from the polar bodies, which constitutes a new animal pole; the two lower macromeres are budding off micromeres of the second quartet.

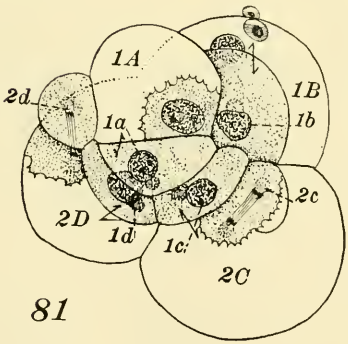
82 (1090) Centrifuged 100 turns of hand machine, fixed 16 hours later. The lower half of the egg is developing normally; the upper half is abnormal and is difficult to interpret.


B
C
1a
1d
1A
1D

79


A
II
B
I
1d
1c
1C
1D
II

80


1A
2d
1B
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1b
2c
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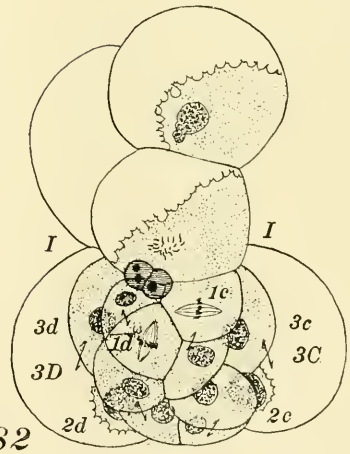
1d
1c
2C

81


I
I
1c
3d
3c
1d
3D
3C
2d
2c

82

PLATE 17

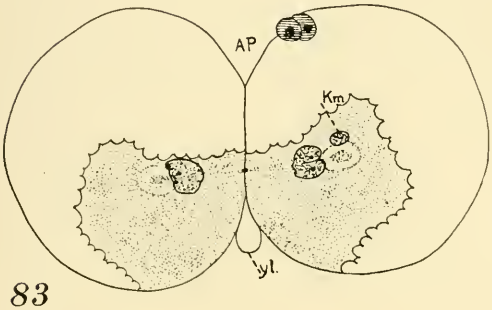
EXPLANATION OF FIGURES

83 (1040) Centrifuged 10 minutes in 1-cell stage, fixed $5\frac{1}{2}$ hours later. Development delayed; cytoplasm and cleavage spindle near vegetal pole. In the right hand cell a karyomere (km) which has not yet fused with remainder of the nucleus. The polar bodies lie a little to one side of the cleavage furrow.

84 (1038) Centrifuged 10 minutes at close of first cleavage, fixed 3 hours later. Axis of centrifuging at right angles to the egg axis. Centrospheres have been moved less than the cytoplasm and nuclei, indicating that they are attached to the surface layer near the animal pole.

85 (1139, 1) Centrifuged $2\frac{1}{2}$ hours in 2-cell stage, fixed at once. The cytoplasm and nuclei are displaced toward the vegetal pole; the centrospheres have been displaced but little from their normal positions and lie between the nuclei and the polar bodies.

86 to 89 (1037) Centrifuged 10 minutes in 2-cell stage, fixed at once. Cytoplasm, nuclei, centrospheres (*Cs*) and spindle remnants have been displaced toward the vegetal pole in figures 86, 87, 89, toward outside in figure 88; the mid body (fig. 86, *MB*) remains unmoved and the centrospheres (*Cs*) lie on the sides of the nuclei toward the polar bodies. The lane of cytoplasm leading from the centrospheres to the animal pole shows that the substances of the egg are not free to move according to their specific weights.



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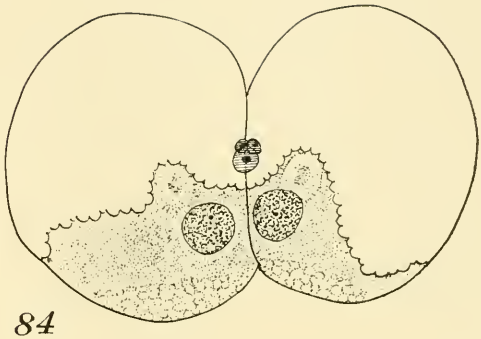

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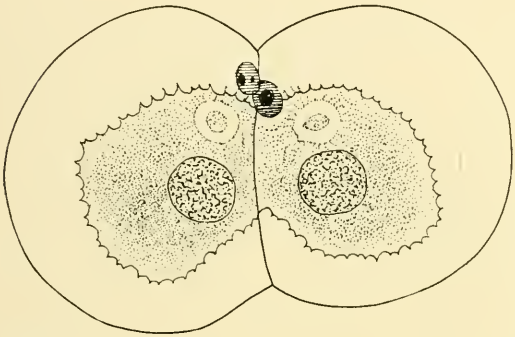

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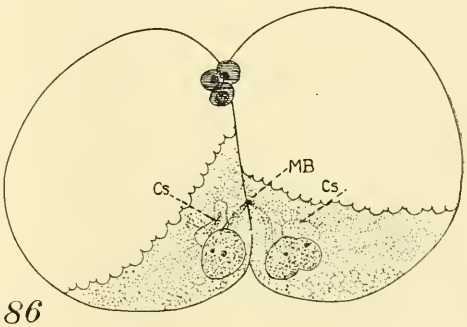

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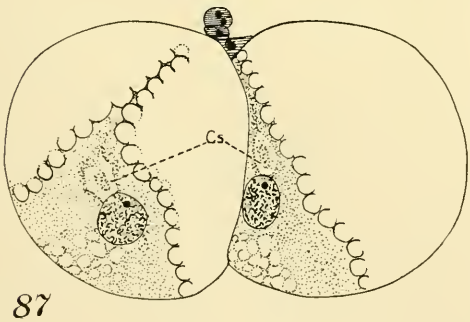

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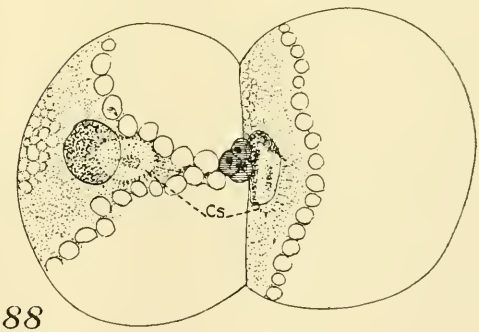


88

PLATE 18

EXPLANATION OF FIGURES

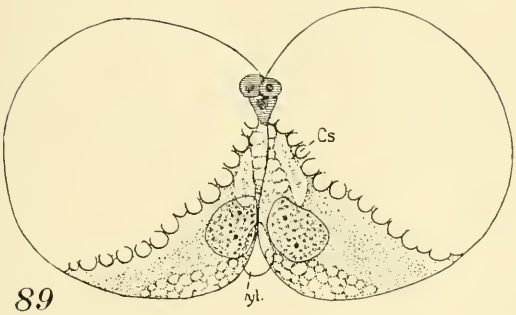
Figure 89 is described on p. 420.

90 (1071) Centrifuged 100 turns of hand machine at 2-cell stage, fixed at once. Centrospheres lie between nuclei and animal pole, with which they are connected by strands of cytoplasm.

91 (1078) Two-cell stage centrifuged 100 turns of hand machine, fixed at once. Axis of centrifuging oblique to egg axis. Centrospheres lie between nuclei and polar bodies.

92 (1148) Centrifuged 4 hours during first cleavage, fixed 18 hours later. Development has been arrested. Cytoplasm, nuclei and centrospheres were displaced toward the vegetal pole. The cleavage furrow has cut in from this pole, leaving one centrosphere in one cell and both nuclei and the other centrosphere in the other cell.

93, 94 (1158) Centrifuged 5 minutes during first cleavage, fixed 3 hours later. Both first and second cleavage mitoses have taken place, but neither cleavage furrow has formed in figure 93, and only one of them in figure 94. The daughter nuclei show traces of division into gonomeres (male and female halves).



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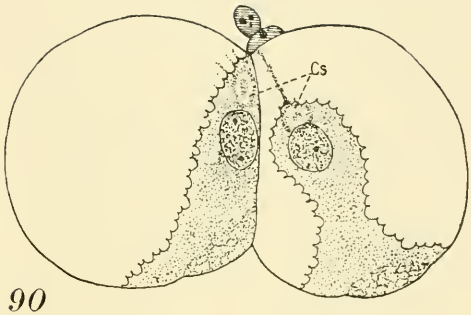

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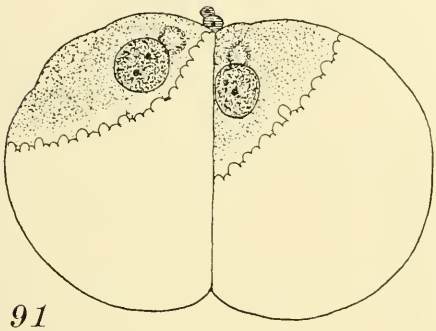

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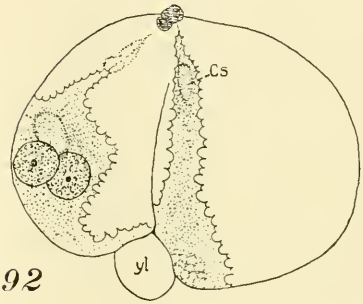

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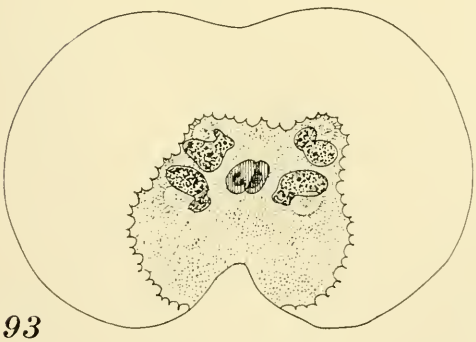

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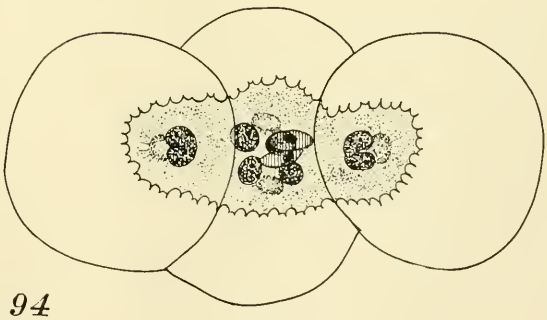


94

PLATE 19

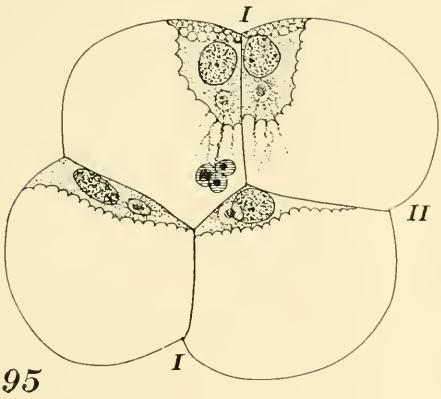
EXPLANATION OF FIGURES

95, 96 (1150) Centrifuged 15 minutes in 4-cell stage, fixed at once. In figure 95 the axis of centrifuging was parallel with the first cleavage plane, in figure 96 at right angles to it. Cytoplasm, nuclei and centrospheres have been displaced from the animal pole by yolk in two of the other cells, but so far as possible the centrospheres remain between the nuclei and the polar bodies.

97, 98 (1038) Centrifuged 100 turns of hand machine during third cleavage, fixed 15 minutes later. The axis of centrifuging was parallel with the first cleavage plane. The spindles are normal though they have been somewhat displaced from their typical positions.

99 (1145) Centrifuged 4 hours in 4-cell stage, fixed at once. Cytoplasm, nuclei and centrosomes were displaced to the vegetal pole, and the third cleavage spindles which were present in the cells are so placed that large yolk-rich micromeres will be cut off at the animal pole.

100 Centrifuged $\frac{1}{2}$ hour, fixed at once. Centrifuging took place during the third cleavage so that two of the micromeres (*1c*, *1d*) were formed at some distance from the animal pole. Lobe (*L*), containing oil, marks the centripetal pole.



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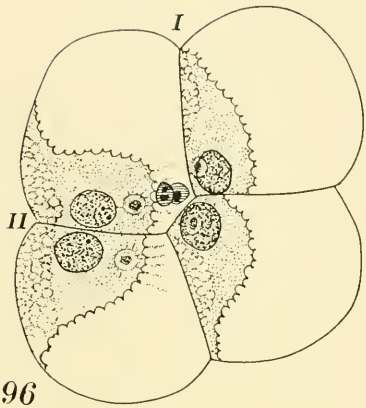

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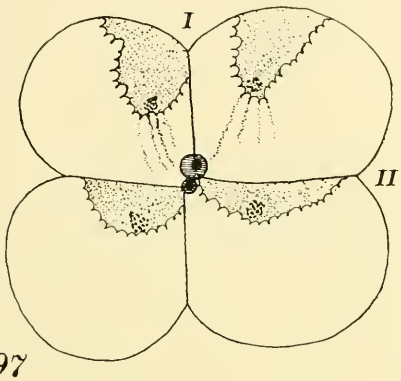

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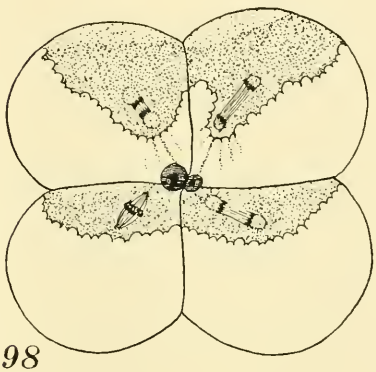

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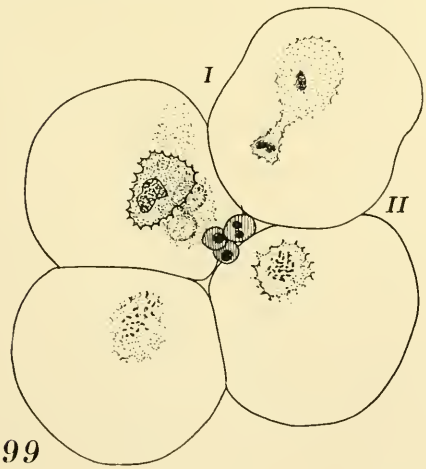

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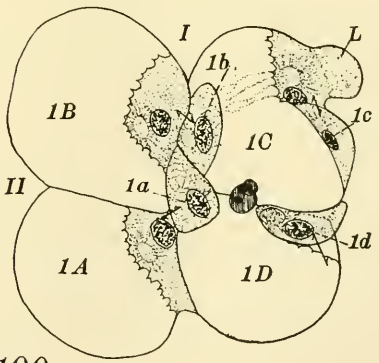


100

PLATE 20

EXPLANATION OF FIGURES

101 (1038) Centrifuged 10 minutes during third cleavage, fixed 3 hours later. Large yolk-laden micromeres were formed at the animal pole.

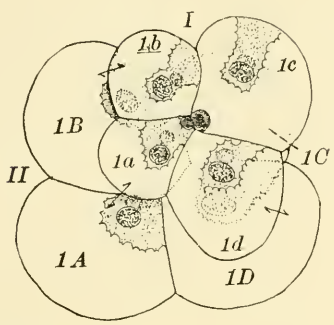
102 (1035) Centrifuged ½ hour during third cleavage, fixed 3 hours later. Egg essentially like the preceding. The micromeres of the first quartet, two of which are dividing, are large and full of yolk, the micromeres of the second quartet contain no yolk and are of normal size and constitution.

103 (1050) Centrifuged 15 minutes, fixed at once. Centrifuged after the formation of the first quartet of micromeres which are not displaced from the animal pole, though the nuclei and centrospheres are displaced in these cells in the same direction as in the macromeres.

104 (1136, 4) Centrifuged 30 minutes during the second cleavage, fixed 12 hours later. The left half of the egg has developed normally, giving rise to two macromeres, and each of these to three micromeres, two of which have subdivided; the right half has divided into a large protoplasmic cell at the animal pole and beneath this a yolk-rich cell which contains two large nuclei connected by a chromatic thread, and four or more centrosomes and spheres, but the cell has not divided.

105 (1139, 2) Centrifuged 2½ hours during the maturation division (?), fixed 18 hours later. Both polar bodies, one of them large and containing a spindle, lie at the pole opposite the protoplasmic cells, which is probably the vegetal pole. The probable identity of the cell is indicated by the labelling and the arrows, but the entire egg is abnormal and hard to interpret.

106 (1129, 1) Centrifuged 10 minutes in gum arabic, fixed 4 hours later. Two eggs fused in the plane of the broken line, each in the 8-cell stage. The polarity of each egg is unchanged by the fusion, as is shown by the positions of the cells relative to the polar bodies.



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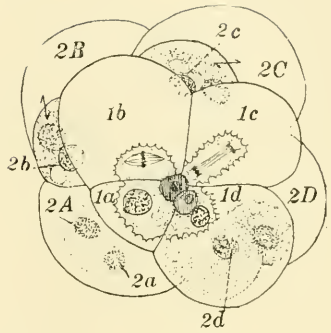

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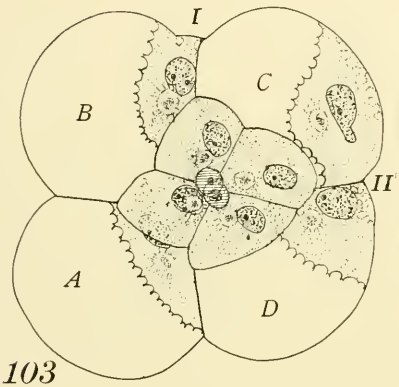

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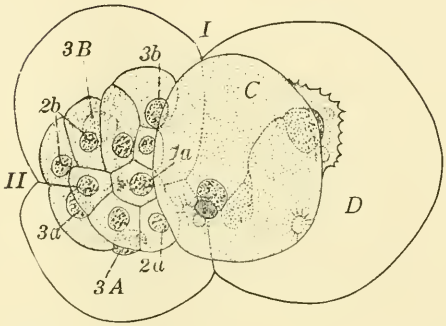

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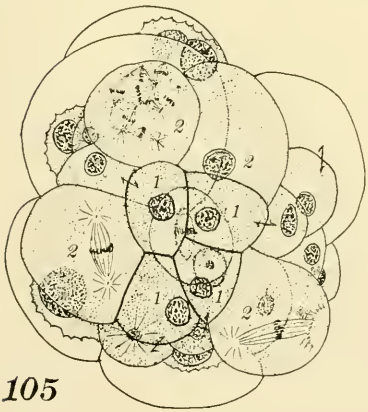

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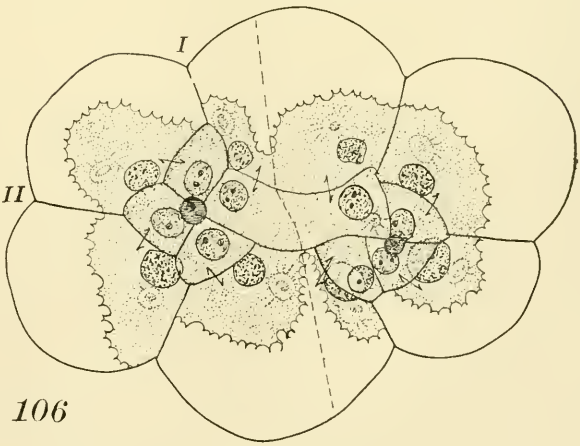


106

PLATE 21

EXPLANATION OF FIGURES

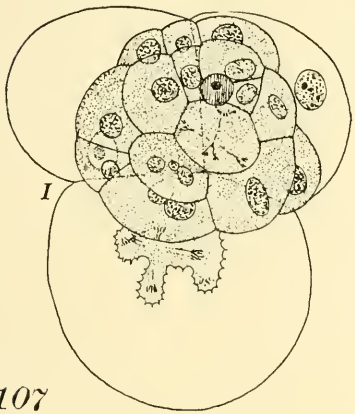
107, 108 (1090) Centrifuged 100 turns of hand machine before or during second cleavage, fixed 18 hours later. The second cleavage was suppressed in one blastomere (the lower) of figure 107 and in both of figure 108, and consequently the micromeres are more or less abnormal in number and position.

109 (1038) Centrifuged 100 turns of hand machine in 2-cell stage, fixed $4\frac{3}{4}$ hours later. The second cleavage was very unequal, with the result that two macromeres are large and two small. There are 18 micromeres, some of which are difficult to identify.

110 (1032, A) Centrifuged $2\frac{1}{2}$ hours during second cleavage, fixed 21 hours later. Egg with four macromeres abnormally joined, and with two separate areas of micromeres, which can not be individually identified with certainty.

111 (1090) Centrifuged 100 turns of hand machine during second cleavage, fixed 16 hours later. Two macromeres are large and two small. There are 24 micromeres, some of which are hard to identify.

112 (1134) Centrifuged 30 minutes in 2-cell stage, fixed 21 hours later. Macromeres arranged in a linear series, micromeres in two groups at opposite ends of the egg.



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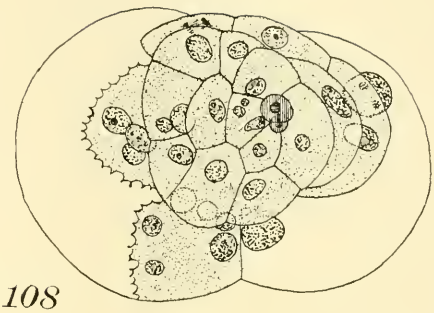

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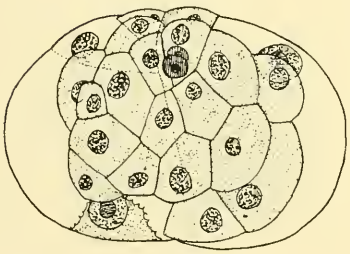

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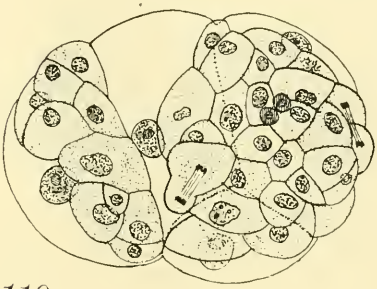

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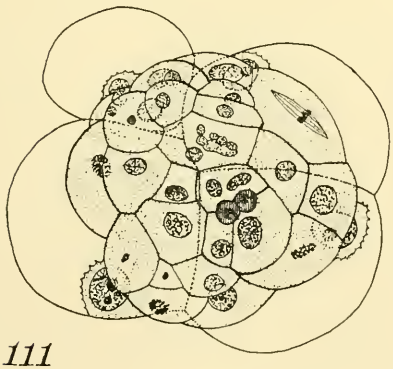

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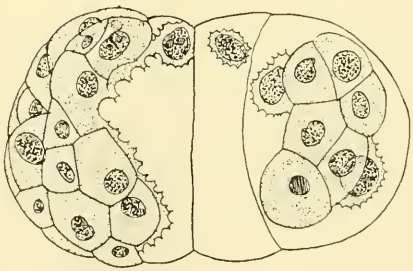

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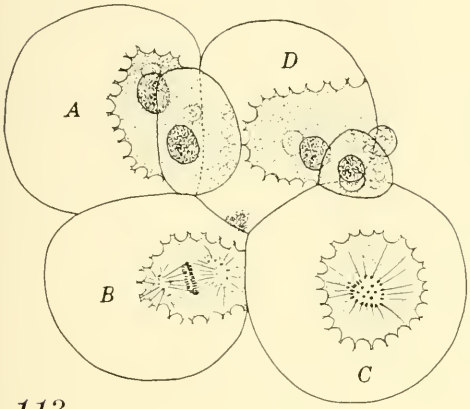
PLATE 22

EXPLANATION OF FIGURES

113 to 118 (1149) Centrifuged 5 hours in the 4–8-cell stage, fixed at once. Figures 113 to 116 are viewed from the vegetal pole, figure 117 from the animal pole. In all these cases the animal pole was centrifugal in position, the vegetal centripetal; consequently the cytoplasm and nuclei of the macromeres was carried to the vegetal pole, where a number of micromeres have been formed, while the first set of micromeres (ectomeres) remains at the animal pole (except in figs. 113, 115, where it had not yet formed). The micromeres at the vegetal pole correspond in number, contents and subdivisions to the second and third sets of micromeres, but it is not certain that they give rise to ectoderm. Figures 117, 118 represent the same egg under a high and low focus; the stippled cells in figure 117 are the first set of micromeres at the animal pole; in figure 118, the second and third sets at the vegetal pole.



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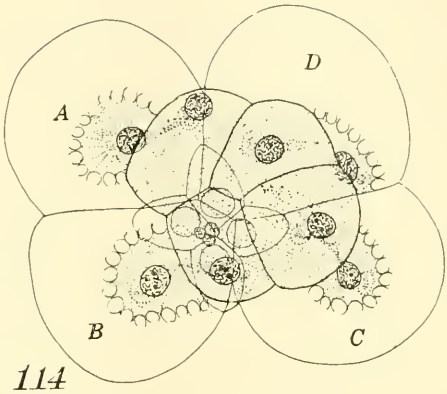

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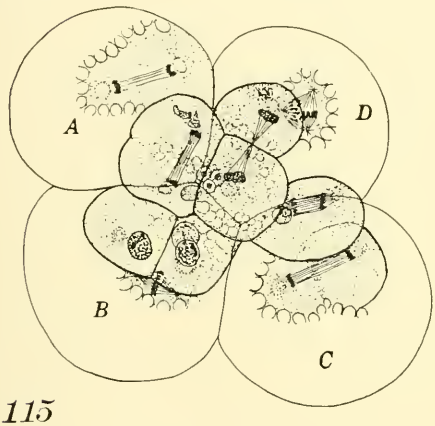

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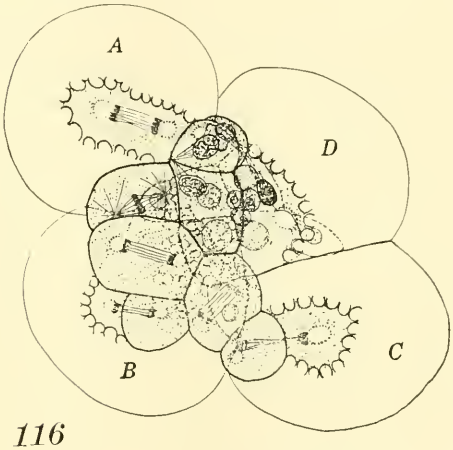

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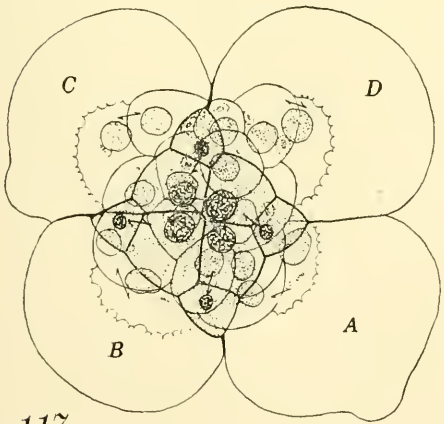

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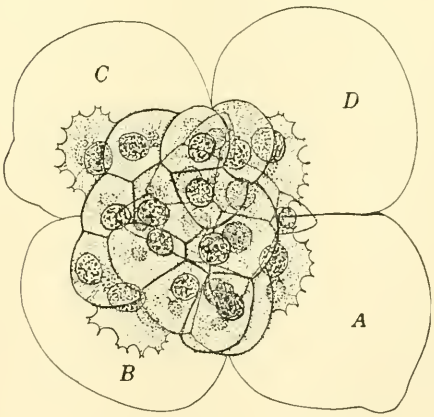


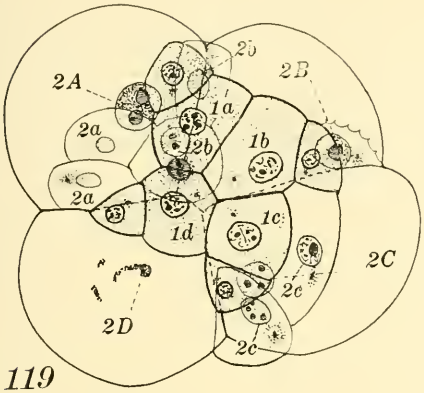
PLATE 23

EXPLANATION OF FIGURES

119 to 122 (1151 and 1152) Centrifuged 5 hours in the 8-cell stage, fixed 5 hours later. In these eggs the animal pole was centrifugal in position, consequently the cytoplasm and the nuclei of the macromeres, *A*, *B*, *C*, *D*, were forced away from the animal pole by the yolk and were held in this new position during one or more cleavages. As a result the second set of micromeres (*2a–2d*) are small protoplasmic cells at the vegetal pole (figs. 199, 121, 122) or they are large yolk-rich cells at the upper pole (fig. 120).

123 (1152) Centrifuged 5 hours in the 2-cell stage, fixed 5 hours later. The second cleavage was forced to form in the equator of the egg, and the first set of micromeres (*1a–1d*) were cut off on one side of the animal pole and are larger than normal. All the macromeres are dividing to form the second set of micromeres (*2a–2d*) which will lie on the animal pole side of those cells. Two cells of the first set (*1a–1d*) are dividing laeotropically as in normal eggs.

124 (1154) Centrifuged 5 hours in the 8-cell stage in the direction shown by the arrow; fixed 24 hours later. The cytoplasm and the nuclei of the macromeres were thrown to the left and toward the vegetal pole and small cells were cut off in this position.



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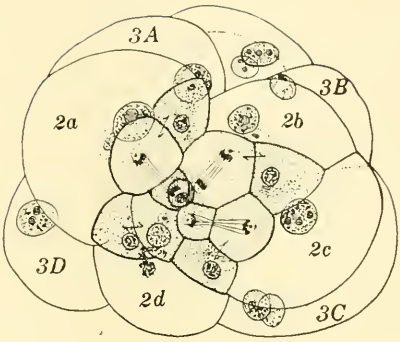

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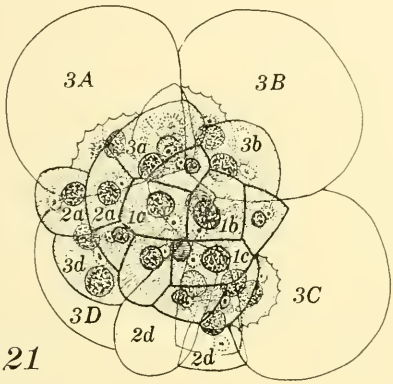

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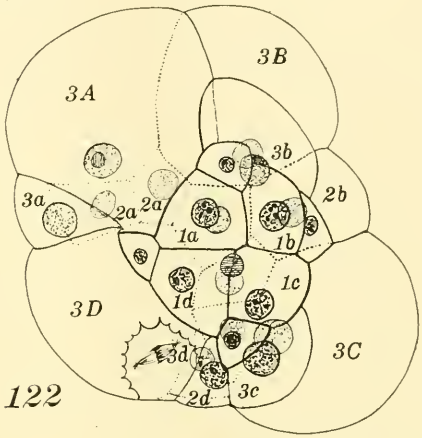

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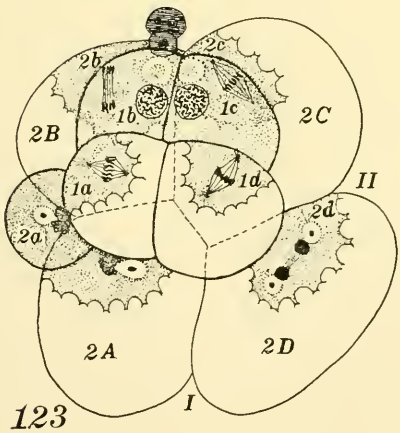

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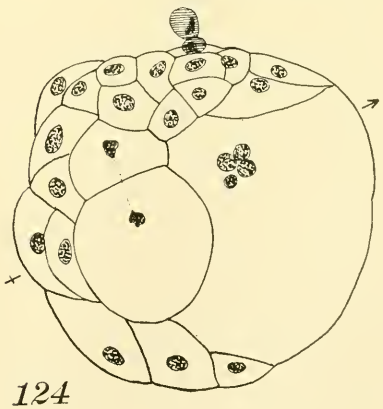

124

THE GROWTH OF PARAMECIUM IN PURE CULTURES OF BACTERIA

GEO. T. HARGITT AND WALTER W. FRAY

From the Zoological Laboratory of Syracuse University

INTRODUCTION

Paramecium has long been a favorite form for studies of various kinds, and with justice since it is of considerable size, is composed of a single cell, is easily obtained, and is easily maintained in vigorous condition in cultures in the laboratory. Some of the factors which are involved in the cultivation of Paramecium have been carefully studied with the result that we have rather complete and precise data on their significance. This analysis includes not only some of the biological factors involved in the activities and functions of the infusoria, but some of the chemical and physical factors of hay infusions and culture fluids.

One of the biological factors of importance is the food of Paramecium. It has long been known that bacteria furnish the chief food supply, and in some of the most careful work attempts have been made to secure a uniformity of food, with some measure of success. It is, however, rather striking that not a single effort has been made by modern methods to analyze the hay infusion bacteriologically. This failure is the more striking in view of the known fact that lack of vigor in cultures and certain 'depression' states, are doubtless due to something unfavorable in the food supply. Also the conclusions of much of the modern work on Paramecium may be significant to the degree that the factor of food is understood, and under control.

Recognizing this gap in our knowledge of one of the most essential factors touching the life of Paramecium we have, during the past year, made a start on a bacteriological analysis of

the hay infusion. More especially we have collected some data on the growth of Paramecium in pure cultures of known bacteria. The work has been most rigidly controlled by checks and tests at all times, which enables us to say that in our experiments the food has been positively known and no foreign bacteria have gained entrance to the experimental cultures.

The data covering the growth of Paramecium are not so extensive as might be desired but certain conclusions seem to be warranted. The technic of the cultures also appears to be of sufficient value to merit attention. Since circumstances have compelled a termination of these experiments for an indefinite time it seems well to set forth the methods employed and the results obtained.

Our thanks are due to Prof. Henry N. Jones of the Department of Bacteriology for the facilities in material and apparatus placed at our disposal in working out the bacteriological part of the investigation.

HISTORICAL REVIEW

The classic investigations of Maupas ('88) were the most extensive and carefully worked out of the earlier attempts to understand the reproductive activities of infusoria. In his paper an account is given of the methods employed, and while these have been considerably modified by later workers they still are of some importance. Among more recent workers Calkins ('02 a) was the first to undertake a careful study of the growth of Paramecium under known conditions and with controlled factors. This paper gives a detailed explanation of the method of making hay infusions, methods of cultivation of the animals on depression slides, and the like. Calkins' chief modification of the earlier methods of study lay especially in growing Paramecium in depression slides with small amounts of liquid, and their isolation each day to prevent conjugation.

As a criterion of the favorableness of the culture media, and as an indication of the rate of growth and metabolism Calkins ('02 b) used the rate of fission. "The division-rate is taken as the measure of vitality, for it represents the rate of metabolism,

growth, and reproduction. A better index of the general vitality could not be found and while fairly constant from day to day, its fluctuations mark out clearly the periods of vigor and depression." The use of the division rate as an index has been accepted by all who have worked on the infusoria. In the cultivation of these animals Calkins used a hay infusion, always made in the same way, and from the same kinds of material. This infusion was raised to the boiling point and cooled before using. The composition of the medium was, therefore, fairly constant, indeed results of later work suggest the possibility of its being too constant. Temperature and other physical factors were either controlled or known, but the bacterial content of the media was entirely unknown. Calkins says: "The bacteria in the hay-infusion constitute the normal food of the Paramœcidæ. Of these, Bacillus subtilis, is, probably, the only one left alive after the infusion is raised to the boiling point, and this organism, therefore, forms the staple article of diet for Paramæcium in culture." It is not certain that Calkins was entirely correct in his inference for, while B. subtilis is resistant to water at the boiling point there are other bacteria which are nearly as resistant and may be unaffected by that temperature. Furthermore there are numerous bacteria which are morphologically like B. subtilis so the exact form could not be known without the use of bacteriological technic. Even if the inference made were correct it would have no significance since air infection of cultures on slides, or in flasks, might radically change the bacterial flora of the media. Hence Calkins was working with an unknown variable so far as food was concerned. Woodruff ('05) used essentially the same methods of cultivation for various infusoria, and was no more certain of the bacterial food of these forms.

Peters ('07 a and b) by careful chemical analyses of infusions added considerably to our knowledge of the composition of such fluids. Concerning the bacteria he said: "I have never determined the specific kinds of bacteria, but I have observed sufficiently to see that practically the same forms are characteristic of the same cycles of the cultures." Doubtless this means he

could tell whether bacilli or cocci were present. His chemical analysis showed some correlation between the acidity of the infusion and the abundance of bacteria. He suggested a probable sequence of bacteria (correlated, perhaps, with certain sequences of chemical change) which influenced or governed the protozoan sequence. From this one might infer a certain kind of bacterial food was essential for particular infusoria. There was, however, nothing in the investigation of Peters which would establish that as a fact.

Jennings ('08) was the first to fully appreciate the importance of carefully considering the bacterial flora of his cultures, though he made no attempt at identification. His method was probably fairly efficient in introducing similar bacteria into all his cultures, though without the use of bacteriological technic this could not be known. Also no attempt was made to prevent infection from the air. He recognized that certain 'bad conditions' of cultures were probably due to injurious bacteria and under these conditions the protozoa decreased in vigor, or even died. If one attempts to account for this lack of vigor or death of infusoria in cultures he must consider several possible causes. It is probable that death may sometimes be due to toxic excretions of the bacteria; there may not be sufficient bacteria to furnish food for the protozoa; or the bacteria present, though sufficient in number, may not be suitable for food so the protozoa die in the midst of apparent plenty. All of these possibilities must be considered in dealing with cultures.

In making the cultural conditions identical in order to compare the division-rates, Jennings says: "To make the conditions of existence the same *it is not sufficient to attend merely to the basic fluid; the bacteria must also be the same.*" This, he insists, is not a mere theoretical consideration but a practical necessity. The introduction of certain organisms may lead to the death of the protozoa in the culture. In order to secure this identity of bacteria in all cultures Jennings washed all his paramecia in two lots of fresh hay infusion, all being washed in the same fluid for the second washing. This he believed would bring into the second wash fluid all the different kinds of bacteria which were

present in the original cultures from which the paramecia were taken. Finally he added a definite amount of this second wash fluid to each culture in which he was growing Paramecium. By this method he hoped to have the same bacteria in all his cultures. Such a method is more cumbersome than to use exact bacteriological methods, and has other serious drawbacks. Jennings could not have any precise knowledge as to whether he had accomplished his aim of securing uniformity of nutritional conditions; he could only assume that he had. A more serious disadvantage is the impossibility of later duplicating the experiment exactly, and this means no other culture could fairly be compared with this one on account of ignorance concerning the bacteria present.

Woodruff in 1909 introduced some modifications into the cultivation of Paramecium on depression slides by making his media from different materials, hay, grass, pond weeds, material from swamps, ditches and the like. He believed in this way he could make conditions more nearly normal, than by the constant use of a similar medium. Calkins' paramecia died out when kept on a constant medium, and Woodruff believed this was due to the uniformity of the medium. Both at this time and later ('11 a) in analyzing the effect of excretion products on reproduction of Paramecium, Woodruff depended on the bacteria carried with the animals to inoculate his culture media. He assumed the bacterial content of the cultures was the same, hence believed the differences observed in the division-rates were due to the different volumes of culture fluid, i.e., to the amount of excretory products of Paramecium itself. But since it is probable that the bacterial flora was not uniform in all the cultures there is a possibility that the excretory products of the bacteria may have been the occasion for the differences in the rates of fission.

Woodruff and Baitsell ('11 c) found a 0.025 per cent solution of beef extract to be a better culture fluid than hay infusion, since protozoa did as well in a constant medium of this solution as in a varied hay infusion. There was, as they admitted, a variation in the bacterial content in their different slide cultures, but they believed this variation was so slight as to be

negligible as a modifying factor in their experiment. In a further attempt ('11 d) to so control the experimental factors as to determine whether rhythms in reproduction were due to environment they still depended upon the chance inoculation of their media by the transfer of the bacteria with the Paramecium at the time of its isolation. Their belief that the variation in the bacterial content was slight may have been true as to numbers of bacteria, but it is probable that the kinds of bacteria may have been, or come to be, decidedly different. In one culture, for example, one kind of bacteria might have got the start and become the predominant kind, while the predominant type in another culture would be different, and such difference might be significant. Whether it was "unnecessary to attempt to 'sterilize' the paramoecia and feed them on pure cultures of bacteria" they certainly could not determine without a trial.

Other work done since this time on Paramecium has not added anything so far as the nutritional factors are concerned. Therefore, from this brief summary it is clear that not a single worker has had any precise knowledge of the bacterial food of Paramecium in experimental work. Furthermore all have entirely neglected the precaution insisted on by Jennings of making the 'bacteria the same,' save Jennings, himself. While it is not to be expected that each worker would laboriously perform his experiments under strict bacteriological methods, it is comparatively simple and easy to use a method like that of Jennings to render the bacterial content of media as nearly the same as possible. The data obtained by us will show that it is not necessary to carry on all of this work with a strict bacteriological technic. But it will be as clearly shown that there is the necessity of securing a uniformity of bacteria in all cultures which are to be compared, and also the desirability of excluding certain kinds of bacteria from the cultures.

METHODS AND OBSERVATIONS

Bacteriological analysis. Two main points are involved in a bacteriological study of the hay infusion: 1) what bacteria are normally present in a healthy hay infusion; 2) what forms

gain the supremacy in fermenting, putrefying, or other abnormal infusions. Under abnormal infusions are included all which for any reason are unfavorable for continued growth or existence of Paramecium and other infusoria. These unfavorable conditions are probably due to bacteria directly or indirectly and the type of bacteria producing such conditions should be known.

One of the first things to determine in a study of healthy infusions is the source of the bacterial infection and obviously there are three possibilities. A given organism may have been present on the hay and by this means gained entrance to the infusion. It may have been present in the water used for starting the infusion, or it may have settled in from the air. To secure the forms present on hay, in the water or air, cultures were made as follows: The standard hay, infusion used by Jennings and Hargitt ('10) was made by allowing 10 grams of chopped hay to macerate in a liter of tap-water. This was then sterilized in an autoclave to kill all the bacteria. By pulling out the cotton plug from the flask, after the fluid was cool, inoculation from the air was made possible. A second flask was prepared in the same way except that 100 cc. less of water was used. After having been sterilized and cooled 100 cc. of tap water was added to furnish the source of inoculation. The addition of this amount of water made the solution standard. The third culture was made by leaving out a little of the hay till after the sterilization of the liquid. When the remaining hay was added the inoculation from hay was accomplished and this medium was brought to the standard. The three flasks contained the same amounts of water and of hay, the water and hay being identical; they differed, therefore, in the source of bacterial inoculation. Since the flasks were plugged with cotton, after having been made and inoculated, any subsequent differences observed would clearly be due to the source of inoculation.

These cultures were analyzed at the end of a few days, at the end of a few weeks, and again after four months had elapsed. In the analysis the ordinary technic of making plates was followed, the infusion being diluted with isotonic salt solution to prevent crowding of the colonies of bacteria on the agar plates.

After a few days the cultures inoculated from the air and from hay showed the presence of many chromogenic bacteria. Most such were yellow but some pink and some red forms were present. The culture inoculated from water showed only a few of these chromogenic bacteria, but they contained forms which imparted a green color to the agar of the plates. This form (Bacillus fluorescens) was also recovered from the hay-infected culture. But very few colonies of a lobose or running type were present on the plates made at this first analysis, a few stellate colonies coming from the hay inoculation. At the end of two weeks the chromogenic colonies were disappearing and white amoeboid colonies were taking their place, Bacillus fluorescens still being abundant. When the analysis was made four months later the chromogenic forms had almost entirely disappeared and many of the colonies were of an amoeboid or arborescent type, this being especially true of infusions inoculated through air and hay.

These analyses demonstrate a distinct succession of bacterial life in a hay infusion that is left undisturbed for a long time. It is quite probable that certain of these bacteria, especially those which appear later in the sequence, are not favorable for the continued vigor and growth of Paramecium. Such forms are seen to gain the ascendency in undisturbed cultures, and this ascendency is prevented, more or less, by the frequent addition of fresh hay and water.

In selecting the colonies on the plates for the production of pure cultures, it was obviously out of the question to test all and still keep the investigation within reasonable bounds. But such a program is unnecessary, since there are certain types of bacteria which are predominant and it is these which have control over the future of the infusion and which must therefore, furnish the greater part of the food for the protozoa. To attempt to get the minor organisms present would have proved misleading in interpreting later results. Particular attention was therefore paid to securing all the predominant types of bacteria in pure cultures.

The bacteria taken from the plates were secured in pure culture and maintained on agar slants in test tubes. From these slants their morphological and cultural characteristics were determined. In every case the examination was carried far enough to secure a good idea of the general character of the organism. Their shape (whether rod, coccoid, or spiral), size and motility were determined in every case. Tests were made for spores by subjecting them to a temperature of 76°C. for twenty minutes. Their reaction to stain was tested with methylen blue, carbofuchsin, and Gram's stain. Their general cultural characteristics were determined as follows; agar was used to determine the kind and amount of growth; beef broth for an examination of motility and the test for indol; litmus milk was used to show whether the organism produced acid, and whether it produced a coagulation and saponification; in dextrose fermentation tubes was observed the power of fermentation and gas production; gelatin stab cultures were made to determine whether the organisms could liquefy the medium, and to show the general character of growth. In some cases other media were used, such as lactose broth, hay infusion agar, potato.

Having secured a good description of each culture they were identified, so far as possible, by using Chester's "Manual of Determinative Bacteriology." In some cases the identification was exceedingly difficult, since the description of these saprophytic bacteria is incomplete. One or two of the bacteria isolated and used for experimentation could not be identified by name.

In the analysis of abnormal hay infusions several protozoan cultures, formerly rich in Paramecium, were found in which fermentation and putrefaction were in progress and in which Paramecium and other infusoria were either absent or few in number and not in vigorous condition. Materials from six such cultures were plated and pure cultures of the bacteria obtained in the same way as for the normal infusions, and their morphological and cultural characteristics determined. A very large number

of different kinds of bacteria were present and the selection of the forms for further study was largely an arbitrary one. Those bacteria were isolated which were present in the largest numbers in the hope that they were the ones causing the abnormal conditions. This is a fairly logical method of choice since only those which are very abundant can be active in producing changes in the infusion. In each of the abnormal hay infusions examined there was a distinctly sour or putrefactive smell at the time of the examination. This odor was used later as a test in determining what bacteria were responsible for the production of the abnormal conditions. Flasks of sterile hay infusion were inoculated with different pure cultures of the bacteria, or in some cases inoculated with bacteria of a certain type but not necessarily of one kind. At the end of a few days, or only after several weeks in some cases, some of the flasks so inoculated gave odors similar to those of the original infusions. The bacteria present in such flasks were later used in pure cultures for feeding Paramecium to further test their unfavorableness as food.

Not all of the bacteria which were isolated were completely studied and identified. But all which were used for experimentation with Paramecium were carefully studied and a complete description of their characteristics will be found in a table at the end of the paper. It may be said in general that the bacteria isolated from abnormal infusions are more zymogenic than are those from normal infusions.

Preliminary experiments. The paramecia which were used for nutrition experiments with the pure cultures of bacteria were obtained in pure strains. The necessity for having all the protozoa of one and the same line is so obvious as to need little comment. In order that results may be comparable the paramecia must be alike so far as possible and those of the same pure strain are as nearly identical as can be obtained. Hence all the animals used in our experiments are descendants of a single individual of Paramecium aurelia or of Paramecium caudatum. In the beginning both P. aurelia and P. caudatum were used but owing to the lower rate of division of the latter, and to its less marked adaptability to growth in depression slides,

the work was later limited to P. aurelia. The results of both are given but those of P. aurelia cover a longer period of time. For a discussion of the differences of the two species the reader is referred to Jennings and Hargitt ('10), and Woodruff ('11 b). In the starting of the pure strains of the Paramecium the single individuals were isolated in the usual way with capillary pipettes and grown in depression slides, or in dishes to produce large cultures.

Before starting the experiments on growth in pure cultures of bacteria the protozoa were grown in depression slides for a time in the usual manner, depending upon chance inoculation of the media to furnish the bacterial food. The purpose of this was to determine the normal rate of fission, to acclimatize the animals to growth in a limited amount of medium, if such was necessary, and to determine what was the best medium for this growth. The slides were used without cover glasses and were kept in moist chambers to prevent evaporation of the fluid, the usual practice for work of this kind. In the initial experiment the medium used was hay infusion liquid taken from a stock culture and filtered to remove the protozoa. This medium was placed in the depression slides and examined with a binocular microscope to be certain that no strange paramecia, and no protozoa of any kind, were present.

The rates of fission for two weeks averaged approximately one per day for P. caudatum and nearly one and a half for P. aurelia. As might have been expected this filtered infusion was found not to be entirely satisfactory. It varied in composition and in age and was sometimes too rich, since the bacteria increased so rapidly as to hamper the activities of Paramecium. The standard 1 per cent hay infusion was also unfavorable if used full strength, and it was necessary to dilute it somewhat. It was obvious that if hay infusion was to be used as a culture medium it must be so standardized in its preparation that its composition did not vary, and also it must be of a concentration suitable for use in the depression slides. To determine what concentration was best the standard infusion was diluted 20 times, 10 times, and 5 times with water making standard infusions of 0.05 per

cent, 0.1 per cent, 0.2 per cent, respectively. At the same time a 0.025 per cent solution of Liebig's extract of beef in tap-water was made.

The sister cells obtained by several divisions of a single Paramecium were distributed between the various fluids and grown for some time, being changed to fresh solutions daily. The results for growth during two weeks are shown in tables 1 and 2.

Tables 1 and 2 show that deaths were frequent in the 0.2 per cent hay infusion, and with both P. aurelia and P. caudatum the results showed the superiority of the 0.1 per cent hay infusion and the 0.025 per cent beef extract solution. From this time on the 0.1 per cent hay infusion was used as the culture fluid for all work. Occasionally the 0.025 per cent beef extract was used and its use will be indicated each time. The hay infusion was used rather than the beef extract solution, in order to have the solutions of the same sort as an ordinary infusion. The objection might be raised that since Woodruff, and Woodruff

TABLE 1

Growth of Paramecium aurelia in mixed cultures of bacteria, in media of different concentrations. Figures represent number of divisions. H. I., hay infusion; B.B., beef extract; [1] *dead replaced from 0.1 per cent hay infusion*

Paramecium aurelia I

	MARCH						APRIL					TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	25	27	28	29	30	31	1	3	4	5	6		
0.05 per cent H. I.	2	4	1	2	2	1	3	1	2	2	1	21	1.615
0.1 per cent H. I.	2	5	1	3	2	1	3	1	2	2	1	23	1.769
0.2 per cent H. I.	2	5	1	0[1]	2	1	2	1	0	1	1	16	1.231
0.025 per cent B. B.	3	4	1	3	1	1	3	1	1	1	1	20	1.538
Filtered infusion	2	2	1	3	1	0[1]	2	2	2	1	2	18	1.384

Paramecium aurelia II

0.05 per cent H. I.	2	3	1	2	2	1	3	1	1	3	1	20	1.538
0.1 per cent H. I.	1	5	1	2	2	1	2	2	2	2	1	21	1.615
0.2 per cent H. I.	2	0[1]	2	2	0[1]	1	2	2	1	2	1	15	1.153
0.025 per cent B.B.	2	4	1	2	2	2	4	2	1	1	1	22	1.692
Filtered infusion	2	3	1	2[1]	2	2	3	2	2	1	0	20	1.538

TABLE 2

Growth of Paramecium caudatum in mixed cultures of bacteria, in media of different concentrations. Figures represent number of divisions. H. I., hay infusion; B. B., beef extract; [1] *dead, replaced from 0.025 per cent B. B.;* [2] *dead, replaced from 0.1 per cent H. I.*

	MARCH						APRIL					TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	25	27	28	29	30	31	1	3	4	5	6		
Paramecium caudatum I													
0.05 per cent H. I.	2	3	1	1	1	1	1	1	1	0	1	13	1.000
0.1 per cent H. I.	2	3	1	1	1	1	2	2	1	0	1	15	1.153
0.2 per cent H. I.	2	4	1	1	1	1	1	1	0	0[2]	2	14	1.077
0.025 per cent B. B.	2	3	1	1	1	1	2	1	1	0	2	15	1.153
Filtered infusion	2	0[1]	0	1	1	1	2	2	2	0	2	13	1.000
Paramecium caudatum II													
0.05 per cent H. I.	1	2	1	1	0	1	1	1	1	0	1	10	0.769
0.1 per cent H. I.	2	3	1	1	0	1	1	1	2	0	0	12	0.922
0.2 per cent H. I.	0[2]	0[2]	1	1	0	1	0	0	0	0[2]	0[2]	3	0.231
0.025 per cent B. B.	2	3	0	1	2	2	2	2	1	0	0	15	1.153
Filtered infusion	2	2	0	1	1	0	1[1]	2	1	0	1	11	0.846

and Baitsell had found a solution of beef extract to be a better medium for constant use than hay infusion, we should have used it here in place of the hay infusion. But the experiments just recorded showed little difference for two weeks, and the above mentioned workers found the beef solution superior mainly when used for extended periods. Our cultures were planned to cover periods of about ten days, long enough to show the effects of pure cultures of bacteria on Paramecium, but not of sufficient length to be affected by the constant use of the hay infusion.

Technic devised for the growth of Paramecium in pure cultures of bacteria. It has just been stated that when the most desirable concentration of hay infusion was found (viz. 0.1 per cent, which is the standard solution of Jennings, diluted ten times with water) this one concentration was used for all subsequent work. A supply of this medium was prepared, placed in test tubes, the latter plugged with cotton and sterilized in an autoclave at a temperature of about 130°C. Sixteen lines of Para-

mecium were started on depression slides in this sterilized medium, and within three days all were dead. Others were started and similar results were obtained. After various tests had been applied to account for this action, it became clear that media such as beef extract and hay infusion were apparently so modified or changed in their composition by the high temperature of the autoclave as to render them unfit for the existence of Paramecium. The bacteria could live in such media but Paramecium invariably died in a short time. Another lot of the 0.1 per cent hay infusion was made, placed in tubes and sterilized by heating in the Arnold Steam Sterilizer on three successive days. In this the temperature never gets above 100°C. In hay infusion sterilized in this manner Paramecium lived satisfactorily and no trouble was experienced after this.

In order to grow Paramecium in pure cultures of bacteria it was necessary to devise a technic, and to organize a plan of checks and controls over bacterial contamination, chiefly from the air. It was in this preparatory work that the greatest difficulty was experienced. As it worked out finally the precautions are not difficult, but they are efficient.

One of the first requirements was to get Paramecium free from all bacteria of any sort. If this could not be done it would obviously be of no avail to make use of pure cultures of bacteria, since they could never be pure after the Paramecium was introduced. Any method which is practical must be expeditious, must rid Paramecium of all bacteria, and must leave the animal in the same active, vigorous condition as at the first. Chemicals and heat can not be used, for Paramecium is more sensitive to these than are bacteria in general. The only method, therefore, must be one of washing in sterile liquids till all the contaminating organisms are removed. Many methods were tried and abandoned as ineffective or impractical. One of the most successful of early attempts was with the centrifuge. A pipetteful of Paramecium was placed in a sterile centrifuge tube and considerable sterile hay infusion added to dilute the bacteria. The paramecia were thrown to the bottom of the tube and as soon as the tubes came to rest the wash fluid was removed and

fresh added with a sterile pipette. This was repeated five times in the same way. Samples of the five wash fluids were then plated in Petri-dishes and the following number of bacteria found present.

TABLE 3

Showing reduction in numbers of bacteria by washing Paramecium through 5 wash fluids in a centrifuge

NUMBER OF WASH WATER	I	II	III	IV	V
Number of colonies of bacteria on the plate..	500	200	50	11	3

This showed that it was possible to reduce the number of bacteria greatly, but the results were not satisfactory; absolute sterility of Paramecium was desired. The method was not very practical since a great deal of time was consumed, the wash waters had to be drawn off immediately after the centrifuge stopped or the paramecia rose in a body and prevented the removal of the wash water. The method has the advantage of permitting the reduction in number of bacteria to approximate sterilization of large numbers of the protozoa at one time, and would be useful for any experimental work which demanded large numbers of the protozoa.

Another serious drawback to the centrifuge method is the difficulty of keeping the wash waters free from contamination by bacteria of the air. The air may contain such enormous numbers of bacteria that instruments and media which are sterile to start with will be contaminated unless precautions are taken to prevent the contact of air bacteria. A sterile pipette laid down on the table is no longer sterile, a wash water left unprotected is soon contaminated by air bacteria. Any successful sterilization method must, therefore, not only rid Paramecium of the bacteria sticking to it, but must prevent the possibility of contamination from any source, air or otherwise.

In the next method many of these difficulties were overcome. A single Paramecium was isolated with a sterile capillary pipette and washed through a number of sterile wash fluids in watch crystals placed inside of sterile Petri-dishes. Under the protecting cover of the dish danger of outside contamination was

reduced to a minimum. It was believed that Paramecium swimming about in the liquid contained in the watch crystal would soon free itself of bacteria, and that the large amount of liquid present would so dilute the bacteria as to reduce the number of washings necessary. However a single Paramecium aurelia swimming in a large volume of liquid in a watch crystal was often lost or else a great deal of time was consumed in searching through the liquid and recovering it. This method was therefore, abandoned.

In the final attempt only small amounts of the sterile medium were used for washing and this was done in the ordinary depression slides enclosed in Petri-dishes. The animal was isolated with a sterile pipette, the cover of the dish raised to permit the entrance of the capillary tube, Paramecium was deposited in the sterile medium of the slide, and the cover of the dish dropped. Paramecium was washed in this way (using a fresh sterile pipette each time) through five different fluids. To determine the reduction in number of bacteria by this washing and whether sterilization was accomplished, each wash fluid was examined bacteriologically. Agar plates were inoculated with a little more of the wash water than would ordinarily be carried over in the pipette when making a change from one slide to another. In the last plate (in these tests) Paramecium itself was introduced together with the wash fluid, so that we might determine whether there were any bacteria still sticking to the animal. After a few days incubation the colonies which developed on the plates were counted. The number of bacteria in each wash fluid is indicated in the following table, which includes the washing in the watch crystals and in the depression slides.

This table shows the advantage of washing Paramecium in depression slides enclosed in Petri-dishes. The animals are at least approximately sterile after the third washing and whatever contaminating bacteria may still be present would certainly be removed by the fourth or fifth washing. The fact that the fifth plate showed no developing bacteria is proof that Paramecium was entirely free from bacteria, for the animal was included in the water that was placed in the fifth plate. Had any bacteria

TABLE 4

Shows the reduction in number of the bacteria by washing Paramecium through 5 wash fluids in watch crystals and in depression slides, when the crystals and slides were enclosed in sterile Petri-dishes

	WASHED IN WATCH CRYSTAL	WASHED IN DEPRESSION SLIDE
Plate I. 1st wash water....	2500 colonies per drop	2000 colonies per drop
Plate II. 2nd wash water...	1500 colonies per drop	49 colonies per drop
Plate III. 3d wash water....	1000 colonies per drop	3 colonies per drop
Plate IV. 4th wash water...	8 colonies per drop	Sterile
Plate V. 5th wash water... (Paramecium also on plate)	1 colony per drop	Sterile

remained on the body of the protozoan they would have been the starting point of colonies of bacteria in the agar around it. It is possible therefore to get Paramecium absolutely free from bacteria by washing in sterile water. The success of this method was so marked that it was used throughout the study in preparing all animals for growth in pure cultures of bacteria.

The complete sterilization process as finally modified may be described as follows: A large number of capillary pipettes (40–50) were prepared by drawing out one end of a glass tube into a capillary tube. A part near the opposite end was heated and drawn out slightly so that a narrowing of the tube was produced at this point. Into this constricted portion of the pipette a small roll of cotton was placed and the pipettes were then sterilized in the hot air sterilizer at a temperature of 160–170°C. The rubber bulb of the pipette was not sterilized since heating, even in the steam sterilizer, destroyed its elasticity. But the plug of cotton in the constricted portion of the pipette effectively filtered out all bacteria which might have been contained in the bulb, so that none could pass into the pipette beyond the cotton. Consequently none of the bacteria in the non-sterile rubber bulb could get into any of the liquids used in the cultures, wash fluids, and the like. Next, depression slides were placed inside Petri-dishes and these were sterilized in the hot air sterilizer for 30 minutes; they were ready for use as soon as cooled without further handling or manipulation. Tap-water was used as the wash fluid and this was sterilized in the autoclave, removed and cooled. When these preparations had been

made the rest of the process could be finished in five minutes. The depression slides were filled with sterile water, and the protozoa were carried successively through five of them. In introducing the sterile water and in changing Paramecium from one slide to the next the cover of the Petri-dish was lifted just far enough to permit the insertion and withdrawal of the capillary portion of the pipette. The chance of bacterial contamination was so slight as to be negligible, being no greater, indeed, than in ordinary bacteriological procedure.

The next test was that of the moist chambers. It is essential to keep the slide cultures in moist chambers in order to prevent evaporation of the culture fluid, but how were we to sterilize our moist chambers and especially was there any chance of maintaining a sterile atmosphere within them? If not, it would be impossible to continue the problem since the slide cultures would soon be contaminated by foreign bacteria and Paramecium would be feeding on unknown, instead of known, organisms. In order to get some idea of the relative numbers of bacteria in a moist chamber under ordinary unsterilized conditions as compared with the number of bacteria in the air of the laboratory, two sterile agar plates were prepared; one was left exposed to the air of the covered moist chamber, the other to the air of the room for two hours. The number of colonies on the plates were counted after two days growth and showed there was but one-tenth the chance of contamination in the chamber as compared with the air of the laboratory. But the plate within the moist chamber had some colonies of bacteria and the chance of contamination of the cultures kept within the chamber was still too great. If such a study is to have any significance the greatest possible pains must be taken to avoid any contamination, if such a thing is possible.

The next plan was to use Petri-dishes as moist chambers. The dishes used were just large enough to contain a single slide. These dishes with their enclosed depression slides were sterilized in the hot air sterilizer and were kept closed till ready to be used. Then with a capillary pipette the depression was filled with the culture fluid containing the strain of bacteria to be used,

and with another sterile pipette some sterile water was placed in the dish to furnish the moist atmosphere. The isolated Paramecium, cleaned from all bacteria by washing in the manner already described, was then introduced by means of a sterile pipette into the culture medium of the slide. This entire process could be accomplished more quickly than the description can be written, and during the process the cover of the Petri-dish was not raised more than enough to permit the entrance of the capillary portion of the pipette. The sterilization of the slides and dishes took some time and the change of Paramecium each day to fresh fluid took longer than if a number of slides were in a single large moist chamber. But it was possible to place the entire Petri-dish on the stage of a binocular microscope and all observations could be made without opening the dish. The chance of contaminating bacteria gaining entrance was therefore reduced to a minimum. Tests showed that, with such precautions, foreign bacteria did not gain entrance.

A further question demanding an answer was the effect of using a small Petri-dish as a moist chamber as compared with the use of large receptacles of the usual type. To answer this, isolated paramecia in slides, with ordinary hay infusion and its mixed flora of bacteria, were kept in large moist chambers and others with sister paramecia were cultivated in identically the same way except they were placed in Petri-dishes as moist chambers. Such parallel cultures showed absolutely no difference, the rate of division was the same, the variation in rate of division from day to day was the same in the two cases, and there was no greater evaporation of liquid in the small than in the large chambers. Such control cultures were run for several weeks, with some of the slides shifted back and forth, and no differences could be detected. It is clear, therefore, that the size of the moist chamber, so far as tested by us, is not a matter of importance. In some respects Petri-dishes make ideal moist chambers, and for certain lines of work would be far better than larger chambers. The Petri-dishes may be piled on top of each other the same as watch glasses and actually require less room than the larger dishes.

Another control was carried on as a check on the accuracy of results and methods. This concerns the determination of the extent to which pure cultures of bacteria in the slide cultures remain uncontaminated for a long period. In every transfer of Paramecium to fresh medium, or in any other manipulation in the Petri-dish moist chamber, sterile pipettes were always used. A pipette once used for one purpose was not used again for that or any other purpose till it had undergone a sterilization in a closed vessel in the hot air sterilizer for a half hour at 170°C. Doubtless this had much to do with the reducing of chances for contamination by bacteria. After Paramecium had been grown in pure cultures of bacteria for 10 days or more, having been transferred to fresh media each day, the last medium was plated on an agar plate, also some of the water in the bottom of the dish was plated. In one such case it was found that only one contaminating colony was on the plate to 350 other colonies of the pure culture used. In other cases, which represent the usual condition, there were no strange bacteria present. Thus at the end of this period in the most extreme cases only one out of 350 bacteria was a strange one; one contaminating bacterium outnumbered in this way can not be considered as seriously modifying the food of the Paramecium. There was no possible question as to whether contamination occurred or not. The morphological and cultural characteristics of the pure culture of bacteria used were well known; in the test any colony with different method of growth, different color, or other difference would at once be noted. If bacteria of the same sort gained entrance from the air it could not be detected, but in such a case there would, of course, be no contamination, i.e., no introduction of a different kind of bacteria.

Under the discussion of the results of growth of Paramecium it will later be noted that from time to time certain of the protozoa were transferred to a new medium or to a new kind of bacteria. In every such case there was first a washing clean of the Paramecium before the transfer was made, and later tests would show that none of the old bacteria had been transferred.

The preparation of the medium and its inoculation with known bacteria was as follows: before the culture was started a large number of tubes were filled with the 0.1 per cent hay infusion and sterilized so the medium used throughout the experiment would be the same. On each day one of these tubes (to be used the next day) was inoculated with the desired kind of bacteria by transferring some on a sterile platinum loop from the pure culture in an agar slant to the hay infusion. This transfer was made in the usual way for such transfers in all bacteriological work and, therefore, involved no contamination from the air. By inoculating a tube of the infusion ahead of time the bacteria got a good start, the medium when shaken would have the bacteria well distributed, and if any chance contamination occurred when the Paramecium was shifted to the new medium the contaminating organism would be only one in an enormous number and would thus have little effect in modifying the diet of Paramecium.

From the above account we believe it is shown that we have been able to devise a technic which is practical and successful. It appears to us that every possible sort of check and control has been applied to test the method and to determine the purity of cultures, and the like. All these were worked out and perfected before the experiments later described were started so that the preliminary cultures were not considered in the data which follows. Furthermore in the transfers and manipulation of cultures and media only one of us (Mr. Fray) took part, so there can be no difference in the matter of skill and method of preparation and handling. All this would seem, therefore, to warrant us in the belief that our results, so far as they go, are without error.

Growth of Paramecium in Pure Cultures of Bacteria. The hay infusion having been analyzed bacteriologically, the various experiments already described having been completed, and an effective and carefully controlled technic having been devised, it was possible to start cultures of Paramecium in pure cultures of known bacteria. Again let it be mentioned that all the protozoa used were of a single pure strain, i.e., all were descend-

ants of a single Paramecium. Furthermore all had been grown in slide cultures of mixed bacteria previous to being transferred to the pure cultures of bacteria.

For the first cultures bacteria I and II were used as the food. These are both organisms which are abundant in normal hay infusions. The table of characteristics of bacteria will give the description of these forms. Both Paramecium caudatum and Paramecium aurelia, washed clean of all bacteria, were introduced into pure cultures of these bacteria. Along with these, other sister paramecia were grown in the same fluids but with a mixed lot of bacteria as food. This mixture was composed of unknown forms and was obtained by inoculation of the medium from a large stock culture of Paramecium. This sort of control was used since it represents the usual or normal conditions under which the infusoria live.

Since there was a possibility that the bacteria might be unfavorable as food, or might excrete substances toxic to the infusoria, it was thought well to run a second control of sterile paramecia in sterile tap water. Other workers have shown that distilled water causes the death of Paramecium in a short time, but the tap-water did not have any unfavorable effect. Since the sterile water contained no bacteria whatever, and since the animals introduced into the water had been washed free from bacteria, there would be no food for Paramecium in these cultures, and they would starve to death. The length of time before this happened would be a check of the behavior of the animals in other cultures. If the experimental cultures died as soon as the sterile water one, this would mean merely that the bacteria present could not be used as food and therefore the animals starved. If they lived longer and divided the bacteria were serviceable as food and their favorableness as food would be indicated by the rate of division and the length of life. If the protozoa in the experimental cultures died sooner than in the sterile water, obviously there were substances of some sort produced by the bacteria which were harmful to Paramecium. In tables 5 and 6 are recorded the number of divisions. under the various conditions mentioned.

TABLE 5

Paramecium aurelia grown in pure cultures of bacteria, in 0.1 per cent hay infusion. Figures represent number of divisions; [1] dead; [2] animal abnormal

FOOD, ETC.	MARCH					APRIL						TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	27	28	29	30	31	1	3	4	5	6	7		
Bacterium I	3	2	0[2]	0	1	0	2	0	1	1	1	11	0.916
Bacterium I			2	0	1	1	1	0	2	(still alive)		7	0.875
Bacterium II	2	2	0	1	1	0	2	0	1	0[1]		9	0.818
Bacterium II			2	0	2	1	1	0[1]				6	0.857
Sterile water	1	0	0	0	1	0[1]							
Mixed bacteria. Several lines, for 12 days, averaged:												21	1.749

TABLE 6

Paramecium caudatum grown in pure cultures of bacteria, in 0.1 per cent hay infusion. Figures represent number of division; [1] deads; [2] animal abnormal in some way.

FOOD, ETC.	MARCH					APRIL						TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	27	28	29	30	31	1	3	4	5	6	7		
Bacterium I	0	0	1	0	0	1[2]	1[2]	0	0[2]	0	0[1]	3	0.250
Bacterium I	3	0	1	0	1	0	2	0	1	0	0[1]	8	0.666
Bacterium II	0	0[2]	0	0	1	1	1	0	1[2]	0	0[1]	4	0.333
Bacterium II	2	0	2	1	0	1	0[2]	0	0[1]			6	0.600
Sterile water	0[2]	0[1]											
Sterile water	0	0	0	0	0[1]								
Mixed bacteria. Several lines, for 12 days, averaged:												13	1.083

The data show that Paramecium will live for some time in the total absence of food, and may even divide when in a medium of sterile water; in the cases recorded 2 days, 5 days, 6 days was the period of life in sterile water. In only one of these cases was there a division, and this must have been due to reserve food in the body, or possibly to the absorption of water. A second conclusion from the records is that the rate of fission is noticeably reduced, considerably in the case of P. caudatum, not so much in P. aurelia. More significant than the reduc-

tion in the rate of division is the early death of the paramecia. In every case where either of these two kinds of bacteria was used alone as food death of the paramecia occurred within two weeks, in some cases within a week. Since these bacteria were present in great abundance in normal hay infusions, and were present in the control of mixed bacteria, and since the protozoa lived longer in these cultures than in sterile water, it seems clear that the lowering of the rate of fission and the early death of Paramecium is not due primarily, if at all, to toxic excretions of the bacteria. If this is true the only possible conclusion is that neither of these bacteria is suitable as food for Paramecium if used alone.

Paramecium caudatum was rather hard to cultivate in slide cultures, even of mixed bacteria, and because of this difficulty and the uncertainty in interpretation brought about by having to replace the cultures which died out this species was no longer used. All records hereafter refer to Paramecium aurelia only.

Paramecium was next grown in pure cultures of other bacteria. The bacteria are designated by numbers for convenience but their names and characteristics will be found in the table at the end of the paper. The bacteria chosen for this second series of cultures were of two sorts: those from normal hay infusions which were but slightly zymogenic, and others taken from abnormal infusions. It was believed that in the slightly zymogenic forms we might find some which would prove to be satisfactory as food, even when used alone (Bacteria III, IV, V, XI, were isolated from normal hay infusions). Those bacteria (VI, VII, VIII, IX, X) taken from abnormal infusions were chosen to determine whether they were favorable or unfavorable as food. Controls were carried on in mixed cultures in every case.

The results show that the bacteria isolated from abnormal hay infusions were so unfavorable for food that practically no growth or divisions occurred in any of them. Death took place rather quickly, in some cases more quickly than in sterile water. Not only is the unsuitability of these forms as food demon-

TABLE 7

Growth of Paramecium aurelia in pure cultures of bacteria, in 0.1 per cent hay infusion. Figures represent the number of divisions; cultures started on different days. [1] *dead. The last four lines of the table were alive at the end of the experiment*

FOOD, ETC.	APRIL															TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25		
Bacterium III	2	2	2	2	0	1	0	1	0	0	0[1]					10	0.909
Bacterium IV			1	0	1	0	0[1]									2	0.400
Bacterium V			3	0	2	0	1	0	0[1]							6	0.857
Bacterium VI			0	0	0	0	0[1]									0	
Bacterium VII										0	0	0[1]				0	
Bacterium VIII										0	1	0[1]				1	
Bacterium IX										0	1	0	0	0	0[1]	1	
Bacterium X								0	0	0	0	0	0	0	0[1]	0	
Bacterium XI			2	0	1	0	2	2	0	2	2	2	0	2	2	17	1.308
Mixed culture		1	2	0	2	2	2	1	0	1	2	1	0	1	1	16	1.143
Bacterium XI; transferred to mixed culture								2	0	2	2	1	0	2	1	10	1.250
Bacterium XI; transferred to beef broth								2	0	0	1	1	0	1	1	6	0.750

strated but it is also clear that there must be produced by the bacteria excretions which are toxic to the protozoa. The behavior of Paramecium in some of these cultures is remarkable, for example the animal lived in the pure culture of bacterium X over a week, was active and apparently quite normal but in the entire period had not undergone a single division. It would appear that in so long a time Paramecium must have fed upon the bacteria in order to remain alive, but the food while not injurious seems not to be assimilated to any marked degree, or growth would have taken place. In the culture of bacterium IX Paramecium divided only once in the week it was living. It is very obvious that the bacteria isolated from abnormal hay infusions are not suitable food for Paramecium, but may in some cases be distinctly injurious.

In the case of bacteria III, IV, V (isolated from normal hay infusions), there is also no question as to their unsuitability as food when used alone, since death of the protozoa occurred in from a week to ten days. The first day or so in these cultures

might stimulate Paramecium somewhat in its growth and fission-rate, but almost at once the divisions dropped below normal, ceased, and death of the protozoan took place.

Bacterium XI is Bacillus subtilis, present on hay and in the air and is so characteristic an organism in hay infusions as to have received the name of hay-infusion bacillus. This is the only one of the bacteria which appeared to be at all favorable as a single source of food. During the period of two weeks recorded in table 7, Paramecium had a higher rate of division in pure cultures of this organism than in the control culture of mixed bacteria. The rate of division was nearly as high, but not quite, as the division rate in control cultures at any time during the two months in which these cultures were carried on. This suggests that Bacillus subtilis is nearly as favorable a food as the usual mixed flora found in all hay infusions; or the conclusion might be drawn that B. subtilis in the ordinary infusion is the chief dependence of Paramecium for food and the other organisms play little part in a nutritional rôle. To test the matter a little further and to find out the possible effect of a change of diet without a change of medium the following cultures were run. At one of the division periods of the paramecia in the pure culture of B. subtilis, one of the cells was transferred to beef extract of 0.025 per cent in which there was a pure culture of the same bacillus. Another one of the cells from the same source was retained in the 0.1 per cent hay infusion but the bacteria were of a mixed and unknown sort, such as are found in all normal hay infusions. In table 7 these new cultures are the last two of the lot and are headed "Bact. XI, transferred to beef broth," and "Bact. XI, transferred to mixed culture." In the mixed culture Paramecium continued to reproduce at about the same rate, or a little faster, while the Paramecium transferred to the beef extract but with precisely the same food, lowered its rate of division and for 8 days had an average rate of about 0.75 per day. One or two experiments are not sufficient to warrant anything definite, but it appears as though the change of medium had a depressing effect, while

the change of food had a slightly accelerating or stimulating effect.

There followed a period of several weeks during which the precautions with regard to contamination from air bacteria were relaxed, and the slide cultures became inoculated with various bacteria by air infection. The slide culture originally in pure Bacillus subtilis (Bact. XI) was one of these and for these several weeks it was permitted to grow in this mixed culture and had a rate of division of a little over one per day. Beginning May 25 one of the animals of this line was again carefully observed and its divisions recorded. Within a few days, at division periods, two other lines were started from this one, by sterilizing the protozoa and placing them in pure cultures of bacteria. One of these lines was returned to the same food it originally had, B. subtilis (Bact. XI), while the other was fed upon Bacillus fluorescens (Bact. III). We thus have three lines derived from the original B. subtilis line all feeding upon different bacteria. The number of divisions noted in these lines in the three weeks following are tabulated in table 8.

The table shows the rate of division of Paramecium in the cultures of mixed bacteria to be 1.143 per day if averaged for the entire three weeks of the experiment, or 1.2 for the first two weeks and 1.0 per day for the last week. Two other paramecia of the same pure line in previous experiments averaged 1.749 divisions per day for 12 days, and 1.143 per day for two weeks, respectively. All of these lines if averaged would give 1.345 divisions per day as a general average for Paramecium aurelia in cultures of mixed bacteria. The original line grown in pure cultures of Bacillus subtilis divided, on the average, 1.214 times per day while for the two weeks recorded here the rate was only 0.6 per day, only about one half as great. This means, then, that the paramecia which had originally fed on B. subtilis continued to do as well and divided as often when placed on a diet of mixed bacteria for three weeks, but fell off greatly when again fed on B. subtilis. Progeny of this same line fed on Bacillus fluorescens did even more poorly, the average rate of

TABLE 8

Growth of Paramecium aurelia in pure cultures of bacteria, in 0.1 per cent hay infusion. Figures represent the number of divisions; [1] *animals abnormal in appearance; all alive at the end of the period recorded*

FOOD, ETC.	MAY						JUNE									TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9		
Mixed culture	1	1	1	1	1	0	1	0	1	3	1	3	1	1	2	18	1.200
Bacillus subtilis (Bact. XI)						0	1	0	1	1	1[1]	0[1]	2[1]	0[1]	0[1]	6	0.600
Bacillus fluorescens (Bact. III)					0	0	1	0	2	1	0[1]	1[1]	0[1]	0[1]	0[1]	5	0.454

FOOD, ETC.	JUNE						TOTAL DIVISIONS	AVERAGE DIVISIONS PER DAY
	10	11	12	13	14	15		
Mixed culture	1	1	1	2	1	0	6	1.000
Bacillus subtilis (Bact. XI)	0[1]	0[1]	2	1	0	1	4	0.666
	Transferred to Bacillus flavescens (Bact. IV)							
Bacillus fluorescens (Bact. III)	0[1]	0[1]	1	0	1	0	2	0.333
	Transferred to Bacillus flavescens (Bact. IV)							

division being only 0.454 per day; this was only about one half the rate of a similar line in a previous experiment (0.909 per day).

Since the paramecia fed on B. subtilis and on B. fluorescens began to appear dark in color as though filled with undigested food (these are marked as abnormal in the table) they were sterilized by washing and both lines placed on a diet of Bacillus flavescens (Bact. V). In the case of the line transferred from B. subtilis to B. flavescens the rate remained the same and the abnormal appearance changed to normal. The B. fluorescens transferred to B. flavescens dropped in rate of division slightly (0.454 to 0.333 per day). The rate of division of the same line of paramecia fed on this same B. flavescens in a previous experiment had been 0.857 per day, though death occurred within a week in that case. In the series recorded in table 8 all of the protozoa were alive at the end of the experiment.

From the data recorded in the various tables it seems clear that cultures of mixed bacteria are, as a rule, far superior as a diet for Paramecium to a diet of any one kind of bacteria. Most bacteria in pure cultures, even when isolated from normal, healthy, hay infusions, were quite unfavorable if used alone. This was true even when such bacteria were present in enormous numbers in healthy infusions and were the predominant types. Only a single kind, Bacillus subtilis, approached a mixed diet as a favorable kind of food; this sometimes seemed to be a better and sometimes a less satisfactory food.

It would therefore appear, under normal conditions, that Paramecium thrives by virtue of the use of a diet of different kinds of bacteria. It is possible that Bacillus subtilis is the chief dependence of Paramecium as food, as some have claimed, but the experimental evidence does not show the superiority of this form over a mixture of different kinds. The probability that different bacteria are usually eaten is in accord with the structure and habits of Paramecium. This infusorian is one of many which has a mouth constantly open, and apparently there is no cessation in the beating of the cilia. Under such circumstances it is hardly conceivable that there is any choice of food, rather all bacteria which are not too large are swept into the buccal groove in the ciliary current and taken into the body. Doubtless some of the bacteria which get into the body are but slightly, if at all, digested and assimilated. If such forms of bacteria should come to be the predominant type in an infusion one would expect Paramecium to decrease in vigor and many of them to die. There is little doubt that some of the 'bad' cultural conditions observed in infusions are due to just this condition of certain bacteria gaining the ascendency and these being so unsatisfactory as food that the animals die.

It should be possible by using pure cultures of bacteria, mixing these known forms in various combinations in sterile infusions and growing Paramecium therein, to secure a mixture which would be better than the ordinary mixed culture obtained by a chance infection of the culture fluid from air, hay, or water.

Such an attempt was a part of our program but we were not able to carry it out, on account of lack of time to give to it.

Any attempt to correlate the characteristics of the bacteria with their favorableness or unfavorableness as food leads to little. From the table of bacteria it appears that a majority of the bacteria which were recovered from abnormal infusions were of a spreading or running type of growth. It was bacteria of this type which produced the putrefactive or fermentative odors when grown in hay infusions, and when used in pure cultures as food for Paramecium the latter soon died. Any culture fluid which has any considerable number of this type of bacteria is therefore apt to be a very unfavorable medium in which to grow Paramecium.

CONCLUSIONS

The principal points brought out by this investigation may be summarized as follows:

1. Bacteria present in hay infusions gain access from the hay, from the water, or from the air. Some forms may be introduced from all three sources, others only from a single source.

2. Both normal and abnormal (fermenting and putrefying) hay infusions were analyzed and the predominant types of bacteria present were obtained in pure cultures by bacteriological methods. The characteristics of some of the bacteria isolated are tabulated in a table at the end of the paper. A total of 30 different bacteria were isolated in pure cultures.

3. The bacterial flora of a hay infusion changes when the infusion is allowed to stand without adding fresh hay and water. Analyses made at the end of a few days, at the end of a few weeks, and again after four months showed a different flora each time. In old cultures colonies with amoeboid or lobose type of growth, and capsulated forms are present in great abundance.

4. Hay infusions and beef extract solutions when sterilized in the autoclave at a temperature of about 130°C. are so modified by the high temperature as to be unsuitable as a culture medium for Paramecium, since in these fluids the animals died in a short

time. If these fluids are sterilized at a temperature of 100°C. they are not so modified and are satisfactory media for the growth of Paramecium.

5. It was found possible to get the body of Paramecium absolutely sterile by washing in sterile water. This washing was done in sterile tap-water in depression slides enclosed within sterile Petri-dishes. A bacteriological examination of the wash waters showed no bacteria present after the third washing, but five wash waters were used in the preparation of every Paramecium for further study. The Paramecium itself when tested by being placed on an agar plate developed no colonies of bacteria showing positively that its body had been entirely freed from these by the washing. Paramecium so sterilized was not injured in any way and suffered no loss of vigor.

6. Sterile Petri-dishes, large enough to contain a single depression slide, were used as moist chambers and it was demonstrated that they are as satisfactory as large moist chambers. Furthermore the Petri-dishes have certain marked advantages as moist chambers over larger receptacles.

7. Pure cultures of bacteria, with Paramecium growing in them, usually remained uncontaminated by foreign bacteria for periods of at least two weeks (they were not tested out for longer periods). The most extreme case of contamination of such a pure culture was at the rate of 1 foreign bacterium to 350 of the pure culture. It is believed that such a contamination is without significance as having effect on the food supply of Paramecium when the cultures are not carried for long periods.

8. Paramecia were grown in pure cultures of bacteria isolated from normal and abnormal hay infusions. In no case was a single kind of bacteria as satisfactory for food as a mixture of many kinds. This was true whether the bacteria came from normal or from abnormal infusions. The bacteria isolated from abnormal infusions were so unfavorable as to cause death of the protozoa in a few days; probably toxic excretions were set free by these bacteria. The only bacteria in pure culture which approached the control mixed cultures as satisfactory food for Paramecium was Bacillus subtilis; this sometimes seemed better

and sometimes worse than the mixed cultures. A mixture of different kinds of bacteria, therefore, seems essential as a diet for Paramecium grown in depression slides; probably the same thing is true for large cultures.

SUGGESTIONS

From the results obtained in this study it is seen that certain precautions are demanded of those who carry on pedigree cultures of Paramecium or other bacteria-eating Protozoa. Some of these are here suggested:

1. Whenever constancy in the matter of food is desired the pipettes used must be sterile. This is not obtained by the insertion of the pipette in boiling water for the spores of some bacteria are resistant to this temperature. The pipette should be sterilized within a closed vessel placed in a hot air sterilizer, or by insertion in the flame of a burner. The pipette must be sterilized each time before being used.

2. Before starting cultures the protozoa should be sterilized. Bacteriological tests show this may be efficiently accomplished with Paramecium by washing through five or six sterile fluids in sterile depression slides enclosed within sterile Petri-dishes. Transfer is to made each time with a sterile pipette.

3. Where different cultures are to be critically compared all the protozoa, after having been sterilized, should be kept in identical culture fluids with identical bacteria present. So far as the present data goes it suggests the inoculation of the culture medium from a normal hay infusion or by chance infection from the air as preferable to the use of pure cultures of bacteria. But the food must be identical and this can only be the case when a single fluid is inoculated and some of this is added to the slides in which the sterile paramecia are to grow.

4. If the demands for uniformity of food are very strict the slide cultures should be kept in a sterile moist atmosphere, protected from the contamination of air bacteria. This condition may easily be met by placing sterile water in sterile Petri-dishes. Transfers of animals or addition of fluid, and the like, should be

made by raising the cover of the dish only sufficiently to permit the insertion of the capillary portion of the sterile pipette. If these precautions are followed the bacteria will remain practically unchanged for a considerable time, so far as the entrance of new forms are concerned.

5. The degree to which these precautions are to be observed will depend upon the requirements for uniformity of conditions. Paramecium seems to keep in good condition and grow best on a diet of different kinds of bacteria and for most work the usual methods are satisfactory. It is only where the needs of the experiment are for the greatest constancy in all environmental conditions that the above refinements are called for.

6. While a mixed diet is best it must not be forgotten that the number of bacteria on the hay and in the air are very great (30 were obtained in pure culture by us, and these are only a portion of those present), and some of these are injurious to Paramecium. The food is a real factor to be considered and it may be controlled as precisely as desired. For critical work chance infection of media, or cross infection of cultures by the transferral of several animals with a single pipette, is not a satisfactory, nor a scientific method.

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Chart showing the morphological, cultural and bio-chemical features of the bacteria isolated from normal and abnormal hay infusions

NUMBER	NAME	MORPHOLOGY AND STAINING					CULTURAL AND BIO-CHEMICAL FEATURES					
		Source	Shape and size	Motility	Spores	Gram stain	Growth on Agar Agar	Beef broth	Dextrose broth	Litmus milk	Gelatin stab	Indol
I	Micrococcus flavus, Flügge	Water	Small micrococcus	–	–	–	Heavy raised yellow; S. moist	Cloudy, no pellicle	–	Reduced, peptonized	G, filiform; L, stratiform	–
II	Bacterium plicatum, Zimmerman	Hay	Short bacterium	–	–	–	Scanty, light yellow	Cloudy, no pellicle	X	Digested, saponified	G, filiform; L, stratiform	–
III	Bacillus fluorescens—non-liquefaciens	Water	Short bacillus	+	–	–	Raised, white; agar turned green	Cloudy, no pellicle	X	Unchanged	G, filiform; L, none	–
IV	Bacillus Raveneli	Air	Large bacillus	+	–	–	Heavy, white, slimy; S. moist	Clear, no pellicle	–	Unchanged	G, filiform; L, none	–
V	Bacillus flavescens, Frankland	Hay	Short bacillus	+	–	–	Raised, yellow	Turbid, pellicle	–		G, filiform; L, none	–
VI	Not identified	Abnormal hay infusion	Higher bacterium	–	–	+	Raised, white, umbilicate	Clear, no pellicle	–	Alkaline (strongly)	G, filiform; L, napiform	–
VII	Bacillus dendriticus, Lustig	Abnormal hay infusion	Short bacillus	+	–	–	Smooth, white, running	Turbid, pellicle friable	A	Alkaline	G, filiform; L, napiform	–
VIII	Bacillus guttatus, Zimmerman	Abnormal hay infusion	Short bacillus	+	–	–	Small, round colonies	Turbid, no pellicle	A	Alkaline	G, beaded; L, napiform	–
IX	Not identified	Abnormal hay infusion	Large bacillus	+	+	+	Heavy, raised, spreading	Clear, thick pellicle	X	Completely saponified	G, filiform; L, stratiform	–
X	Not identified	Abnormal hay infusion	Short bacterium	–	–	–	Thin, raised, spreading	Turbid, no pellicle	A	Acid, reduced	G, echinulate; L, napiform	–
XI	Bacillus subtilis	Hay and air	Large bacillus	+	+	+	Raised, white, wrinkled	Turbid, pellicle	X	Coagulated, peptonized	G, feathery; L, stratiform	–

G = growth. S = surface. L = type of liquefaction. A = alkaline. X = acid; no gas

+ = motile, spores present, gram positive. – = non-motile, no spores, gram negative, dextrose broth not fermented, indol none.

THE EFFECT OF LONG-CONTINUED HETEROZYGOSIS ON A VARIABLE CHARACTER IN DROSOPHILA

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TWO FIGURES

The belief that a factor may sometimes be contaminated by its allelomorph when the two meet in the hybrid has been upheld by Castle and by some geneticists of the non-Mendelian camp. On *a priori* grounds there is no reason why this might not occur, but there is no evidence for arriving at such a conclusion. The bulk of Mendelian inheritance seems to show that factors are not affected by their allelomorphs.

Bateson supposes that some cases of multiple factors are due to fractionation and that the products of this 'quantitative disintegration' segregate independently of one another. He explains these supposed fractional degradations as due to irregularities in the segregation of the factors in the germ cells, during cell divisions in which he imagines the qualities to be sorted out each to its place. In such a case a character might become weaker and weaker as a result of continued crossing to other stocks even though it originally differed from these stocks in only a single factor. The aim of the experiment considered in this paper was to contribute evidence in regard to the constancy of factors in a state of heterozygosis; it was believed that the apparent fluctuation in factors, which is thought by certain workers to be contamination or some sort of quantitative disintegration, can be accounted for on other and more satisfactory grounds.

The subject of the present investigation is a variable wing character in Drosophila ampelophila which is called balloon, and the factor for which lies in the second chromosome.

The balloon factor was kept heterozygous for at least fifty generations, covering a period of nearly three years. Heterozygous males having the mutant factors streak,[1] dachs, jaunty, curved, and balloon in one chromosome, and the mutants black, purple, vestigial, arc, and speck in the other, were repeatedly crossed to homozygous females containing black, purple, vestigial, arc, and speck. All these mutant factors are recessive except streak, so that the male offspring containing the first named combination of factors were easily picked out. Since there is no crossing over in the male and the females used were pure for *b-p-v-a-sp*, the factors of each kind of chromosome were kept together, and there were only two kinds of males and females produced in each generation, of the same types as those above described. One kind showed the character streak, a marking on the dorsal side of the thorax, and in the other kind this character was absent but the characters of *b p v a* and *sp* were manifested. The males containing streak thus always contained the factors for balloon wing and for its normal allelomorph. The close association of these allelomorphs over such an extended period furnished an excellent opportunity for contamination if contamination really occurs.

Before the balloon character could be studied it was necessary to separate it from the other mutants and obtain flies homozygous for balloon. This process of purification was a somewhat complicated one. The accompanying diagram will serve to illustrate the method of 'freeing' the balloon factor. Males of the heterozygous type just described were crossed with normal females. The F_1 offspring were heterozygous, some containing the *S d j c ba* chromosome and the normal chromosome, others containing the *b p v a sp* and normal chromosomes. The latter were eliminated. Males and females of the former type were then crossed with each other. Part of the offspring (F_2) from this cross received from their mother balloon but none of the other mutant factors (the balloon having crossed over), and from their father they received the chromosome containing *S d j c b a*. These

[1] The factor streak was introduced in the way described only during the later generations.

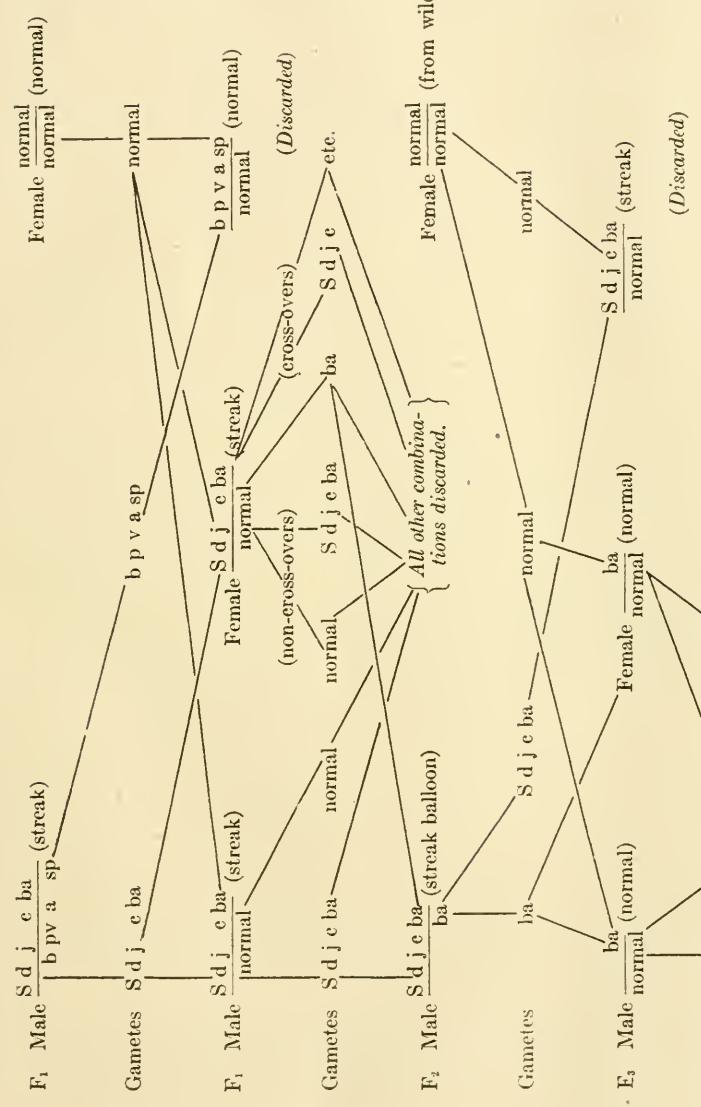

F1 Male S d j e ba / b pv a sp (streak)
Female normal / normal (normal)
Gametes S d j e ba
b p v a sp
normal
F1 Male S d j e ba / normal (streak)
b p v a sp / normal (normal)
(Discarded)
Gametes S d j e ba
normal
(non-cross-overs)
(cross-overs)
S d j e ba
ba
S d j e
etc.
F2 Male S d j e ba / ba (streak balloon)
All other combinations discarded.
Female normal / normal (from wild stock)
Gametes ba
S d j e ba
normal
E3 Male ba / normal (normal)
Female ba / normal (normal)
S d j e ba / normal (streak)
(Discarded)
Gametes ba
normal
F4 ba / ba (balloon)
All other combinations discarded.

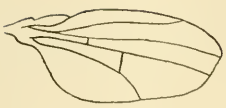
Figure 1

flies were homozygous for balloon but heterozygous for the other factors and they were identified by the phenotypic appearance of the balloon and streaked factors. The other combinations were discarded. Next, males of this type $\frac{S\ d\ j\ c\ ba}{ba}$ were crossed to wild (normal) females in order to eliminate the *S d j c*. Half of the F_3 flies received from their father the chromosome with *S d j c ba* and half received the chromosome with *ba*. Both types of course received from their mother the normal chromosome. The two sorts of flies could be distinguished by the presence or absence of the character streak. Those flies containing streak were discarded. The F_3 males and females that were normal in appearance were bred together and their resulting offspring (F_4) that showed phenotypic balloon were homozygous for this character and contained no other mutant factors.

Now that this balloon factor which had been in a state of continued heterozygosis was freed, it remained to be compared to the balloon in flies which had been kept homozygous for a considerable length of time. The flies used for this comparison had been kept homozygous for an even longer time than the others had been kept heterozygous, the latter having in fact been derived from this homozygous stock.

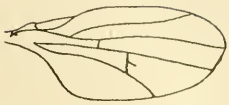
The first problem that was encountered here was to arrange the variations of the character in a consistent series. After a large number of wings had been observed seven grades were established which approximately accounted for all the varieties that had appeared. The accompanying diagrams (fig. 2) will serve to show the nature of these grades, which are based upon the wing venation. The sorts of variation used as standards for these classes were fairly typical, and although all intermediate conditions were found there was seldom doubt in deciding to which grade a particular wing belonged. The different grades may now be considered in detail.

1. Some few wings showed no modification whatever; these were classed as normal. In all others the balloon character made its appearance near the posterior margin of the wing by an

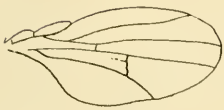


1 normal

2 slight

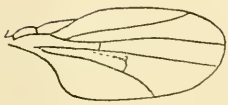


2 slight

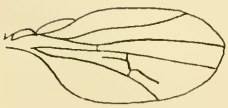


2 slight

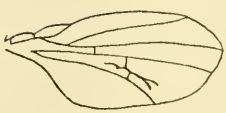
3 moderate



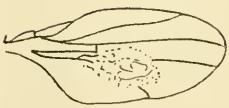
3 moderate



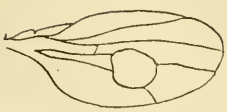
4 marked



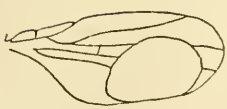
4 marked



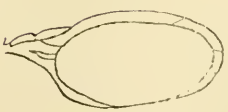
4 marked



5 small blister



6 medium blister



7 large blister

Figure 2

abnormality in the region of the cross-vein which connects the fourth and fifth longitudinal veins.

2. If the modification was merely a bend in the cross-vein, it was classed as 'slight.' Likewise if the disturbance took the form of a slight projection, either proximal or distal, it was considered in the same class, or if the extra mark was apparently disconnected or removed from the cross-vein.

3. If short projections or markings appeared, both proximally and distally, the wing was classed as 'moderate.' Also if only one projection occurred and this was more extended than those of the class 'slight,' even so much as to become branched, it was recorded in this grade.

4. If fairly extended projections in both directions from the cross-vein appeared, the wings were graded as 'marked.' Such projections were frequently branched, especially distally. Wings in which the venation in this region was confused or considerably disturbed were classed in this lot.

5. If a small watery blister appeared in this region of modification which was considerably less than half as long as the entire wing, it was placed in the class 'small blister.'

6. When the blister was approximately half as long as the wing, it was classed as 'medium blister.'

7. The grade 'large blister' was established for a similar condition that occupied the entire or nearly all of the wing surface.

The type and the range of each class being fixed, the question next occurred as to what their order or relative degree of deviation from the normal might be. After a comparison of the amount of modification in each class, the order in which they have been described was adhered to, and experimental support for it was also found and will be described presently.

The method of observing and recording the variations was carefully considered so as to avoid bias. The flies of either the stock (of homozygous ancestry) or of the outcrossed type (of heterozygous ancestry) were inconspicuously marked by amputating a small portion of one member of the front pair of legs. The front pair were chosen because these legs usually project backwards beneath the body rendering them less obvious, and

secondly because these were most easily accessible. The 'branded' flies were thoroughly mixed with those to be compared with them before the record was taken. Each wing was first graded and then identified as stock or outcrossed type. In this process of classification a diagram of the grade types was constantly referred to so as to make the judgment as uniform as possible. Each wing was recorded separately, there being no satisfactory way in which to determine the variation of the fly as a whole.

As far as possible the crosses for comparison were started on the same day, using food from the same batch, of the same age and consistency, and the bottles were placed in the same conditions of environment. Approximately the same amount of food was used in each case; and with the exception of a few instances the same number of parents were allowed to breed in each bottle and have access to this food.

The food and rearing problem was without doubt the most difficult question encountered. It was necessary to start many crosses in order to insure a sufficient number of offspring. Unfortunately, owing to the infertility of the flies, it was often impossible to obtain counts on the same date from stock and outcrossed bottles that had been started simultaneously and with similar food. But the results include observations on a number of bottles and are consistent enough to show that variations in the character due to environmental differences between the bottles were not responsible for the main outcome of the comparison.

The flies for each day were recorded in a correlation table, one mark representing both wings, the modification in the right wing being placed in its proper column within the row labeled for the modification of the left. The data obtained in this way gave a correlation between the right and left wings which justified the order in which the grades had previously been classified. For example, that 'small blister' is a greater deviation from normal than is 'marked' is proved by the fact that 'small blister' (in one wing) shows a greater tendency to be associated with 'marked' (in the other wing) than with 'moderate.' This may be seen by consulting table 1. Similarly each of the other

TABLE 1

		RIGHT WING							
		Normal	Slight	Moderate	Marked	Small blister	Medium blister	Large blister	Totals
Left Wing	Normal	122	28	1	0	0	1	0	152
	Slight	14	190	38	4	1	3	0	250
	Moderate	2	53	130	35	8	4	3	235
	Marked	0	5	23	204	36	20	20	308
	Small blister	0	2	6	30	77	36	17	168
	Medium blister	0	0	3	20	28	36	4	91
	Large blister	0	1	1	12	21	3	3	41
Totals		138	279	202	305	171	103	47	1245

grades may be proved to have the order in the series which has been assigned to it. Exception must be made in the case of the last grade, 'large blister,' but in this case the numbers are not large enough to be significant, and it seems fairly obvious that the 'large blister' represents a greater deviation from normal than medium blister (especially since medium blister is shown by the table to be less normal than small blister).

In table 2 the results for both right and left wings have been added together, but the counts for the stock and outcrossed types are shown separately.

All counts produced by bottles that were started on a certain date (with similar food) are grouped together, with their results for each day shown on a separate line. It should be stated here that the offspring were kept one day after being taken from the bottle, in order to have both types of approximately the same age when graded.

It will be seen from these figures that *the outcrossed type which has been kept in a state of continued heterozygosis deviates even further from normal than does the stock.* The average grade of the former is 4.2, which places this type in 'marked,' and the average grade of the stock is 3.0, or 'moderate.'

The figures show that in twenty-five cases the stock is weaker than the outcrossed type. In only five cases is the outcrossed weaker than the stock, and in one case both are of the same value.

In four of the six latter cases the number of outcrossed flies (2, 4, 1, and 3) was too small to be of significance. In the other two cases the number was somewhat larger than in the above, although still rather small, but here there is very little or no difference between outcrossed and stock (—0.2 and 0).

By comparison of the lots that were started on different days in either stock or outcrossed, it is evident that there is variation in the character (presumably due to environment) quite apart from any difference between the stock and the outcrossed. The differences between the latter two types, however, are too consistent to be due to chance operations of the same cause.

Neither is the difference due to contamination from the normal allelomorph, otherwise the outcrossed would be nearer the normal than the stock is. We find, on the contrary, that the outcrossed type is really better balloon than the stock. Moreover, the standard deviation of the stock is 1.55 grades, and of the outcrossed is 1.37. Hence *there has been no increase in the variability in the outcrossed.* The greater variability of the stock constitutes additional evidence that there has been no contamination or fractionation.

The difference, then, must be due either to variation in the balloon factor itself (multiple allelomorphs) or to a difference in modifying factors (multiple factors). Assuming that variation of one type or the other could occur at all, natural selection would tend to cause the races finally to differ in the direction found. For the wings that made for increased balloon, being less adequate for efficient flight, would tend to be eliminated. Also the watery blister, which usually bursts, often causes the fly to adhere to the side of the bottle and perish. The inbred stock has had an excellent opportunity to become changed in the direction of normal in the period of three years, but the outcrossed did not have a chance for such selection, since balloon is recessive and was kept hidden while in the state of heterozygosis.

Tests could be made to determine definitely whether the change was due to multiple allelomorphs by the use of linkage experiments, since the knowledge which has already been obtained of

A. Stock

DATE STARTED	DATE GRADED	NORMAL	SLIGHT	MODERATE	MARKED	SMALL BLISTER	MEDIUM BLISTER	LARGE BLISTER	TOTAL	AVERAGE
April 30	May 15									
	May 16									
	May 17									
	May 18									
	May 19									
	May 20									
	Total									
May 1	May 15	2	18	26	6	3	1	2	58	3.0
	May 16	2	5	1	1	4	1	0	14	3.3
	May 17	2	6	2	1	1	0	0	12	2.4
	May 18	0	4	2	5	2	0	1	14	3.6
	May 19	0	2	1	7	4	0	0	14	3.9
	May 20	2	2	0	0	0	0	0	4	1.5
	Total	8	37	32	20	14	2	3	116	3.1
May 2	May 15	9	29	11	10	6	3	0	68	2.7
	May 16	19	63	23	19	2	3	3	132	2.5
	May 17	28	51	15	20	4	5	1	124	2.5
	May 18	19	19	11	6	0	1	1	58	2.1
	May 19	15	18	8	7	5	2	5	60	2.9
	May 20	12	13	8	5	4	3	1	46	2.9
	Total	102	193	77	67	21	17	11	488	2.6
May 3	May 15	12	15	29	24	2	0	0	82	2.8
	May 16	34	43	39	19	4	3	0	142	2.4
	May 17	14	39	16	14	3	5	1	92	2.5
	May 18	6	29	9	17	2	0	1	64	2.7
	May 19	16	22	13	17	1	4	1	74	2.7
	Total	82	148	106	91	12	12	3	454	2.6
May 5	May 18	0	6	17	15	11	12	5	66	3.4
	May 19	0	8	6	1	3	0	0	18	2.9
	May 20	0	4	4	3	4	2	1	18	3.9
	May 21	0	3	4	7	4	0	0	18	3.6
	May 22	0	2	1	6	1	0	0	10	3.6
	May 23	6	2	2	1	5	0	0	16	2.8
	May 24	0	0	0	1	7	2	0	10	5.1
	Total	6	25	34	34	35	16	6	156	3.8
May 6	May 18	8	3	44	3	1	2	1	22	2.8
	May 19	7	9	7	2	2	2	3	32	3.0
	May 20	2	7	10	1	1	0	1	22	2.8
	May 21	3	3	3	4	0	0	1	14	2.9
	May 22	4	1	1	2	0	4	0	12	3.4
	May 23	1	2	1	13	4	4	1	26	4.2
	May 24	0	0	0	3	0	0	1	4	4.7
	Total	25	25	26	28	8	12	8	132	3.2
May 13	May 24	12	0	10	13	31	14	4	84	4.2
	May 25	2	6	7	11	2	4	0	32	3.2
	May 26	4	5	13	12	5	6	1	46	3.4
	May 27	10	23	25	24	1	11	4	98	3.3
	May 28	9	7	16	16	4	4	4	60	3.4
	May 29	0	0	0	2	5	1	2	10	5.3
	Total	37	41	71	78	48	40	15	330	3.7
Grand Total		260	469	346	318	138	99	46	1676	3.0

B. Outcrossed

NORMAL	SLIGHT	MODERATE	MARKED	SMALL BLISTER	MODERATE BLISTER	LARGE BLISTER	TOTAL	AVERAGE	DIFFERENCE (B–A)
0	4	17	31	4	1	1	58	3.7	
1	3	6	5	1	0	0	16	3.1	
3	13	14	27	2	1	0	60	3.2	
2	0	3	0	9	4	0	18	4.4	
0	0	6	2	5	3	2	18	4.6	
0	0	0	2	1	1	0	4	4.7	
6	20	46	67	22	10	3	174	3.6	
0	3	1	5	1	1	1	12	3.9	0.9
0	0	1	4	0	1	0	6	4.1	0.9
0	2	0	2	0	0	0	4	3.0	0.6
0	0	1	3	2	0	0	6	4.1	0.5
0	0	1	3	2	0	0	6	4.1	0.2
0	5	4	17	5	2	1	34	3.9	0.7
2	0	0	2	2	0	0	6	3.3	0.6
1	1	2	0	0	0	0	4	2.2	−0.6
0	0	1	1	0	0	0	2	3.5	1.0
0	2	0	0	0	2	0	4	4.0	1.9
3	3	3	3	2	2	0	16	3.2	0.6
0	0	0	1	0	0	1	2	5.5	2.7
0	0	0	0	2	0	0	2	5.0	2.3
0	0	0	1	2	0	1	4	5.2	2.6
2	5	1	16	7	5	2	38	4.1	0.7
1	8	5	12	6	4	0	36	3.7	0.8
0	1	2	4	4	2	3	16	4.8	0.9
1	1	0	5	1	0	0	8	3.5	−0.1
0	0	2	8	0	2	0	12	4.0	0.4
0	0	1	8	4	4	3	20	5.0	2.2
0	0	0	2	0	0	0	2	4.0	−1.1
4	15	11	55	22	17	8	132	4.2	0.4
3	1	2	30	29	13	4	82	4.6	1.8
0	2	4	17	12	5	2	42	4.4	1.4
0	0	4	2	13	4	7	30	5.2	2.4
1	1	0	15	17	8	4	48	4.8	1.9
0	0	0	1	7	4	0	12	4.9	1.5
0	0	0	4	8	7	1	20	5.2	1.0
0	0	0	2	2	2	0	6	5.0	0.3
4	4	10	71	88	43	18	238	4.8	1.6
0	1	3	7	4	3	0	18	4.2	0.0
6	5	6	11	4	0	0	32	3.0	−0.2
3	1	9	14	24	3	6	60	4.4	1.0
3	3	3	25	10	8	2	54	4.2	0.9
0	1	1	33	15	5	3	58	4.5	1.1
0	0	2	1	2	1	0	6	4.3	−1.0
12	11	24	91	59	20	11	228	4.2	0.5
29	58	98	305	200	94	42	826	4.2	1.2

linkage groups in Drosophila is adequate for this purpose. It is important to know whether this difference is due to multiple allelomorphs or multiple factors since if it were found to be due to multiple allelomorphs the further question would be raised as to whether it was due to one or two mutations in the factor for balloon or whether it was due to natural selection working on continual fluctuations in this factor. There is no evidence at present to show whether the latter is true, but this question too could be determined by certain breeding tests.

Another experiment was made with the curved wing character in Drosophila, which was treated in the same general way. The factor curved (*c*), like balloon, was in the chromosome *S d j c ba* that had been subjected to the long-continued outcrossings. After it was purified by a series of crosses in a manner similar to those made in 'freeing' balloon, it was compared to stock which had been kept homozygous for an extended period. Owing to the infertility of the curved winged flies the number of resulting offspring was small. Nevertheless the results corroborate those already given for the balloon character. The character curved appears as a convexity throughout the general surface of the wing and the grades are based on the degree of variation found. After careful observation 4 grades of intensity were established, which may be called: (1) 'Slight curvature,' (2) 'moderate curvature,' (3) 'fair curvature', and (4) 'marked curvature,' and in addition there were also found a number of cases which had to be grouped in a fifth grade, (5) 'spongy wing.' In this experiment there were only two strictly comparable lots of stock and outcrossed flies (made up and counted on the same day). Owing to the small numbers of stock offspring, an extra lot of stock flies started on a different day was counted.

Table 3 shows the correlation between the right and left wings in the case of curved, as table 1 did for balloon.

Table 4 gives the values obtained for the different lots of flies examined. In these examinations, as in the case of the balloon, the flies of one kind were marked and then mixed with those of the other kind. The grades of the wings were then determined before they were identified as stock or outcrossed.

TABLE 3

	SLIGHT	MODERATE	FAIR	MARKED	SPONGY	TOTALS
Slight	4	2	0	0	0	6
Moderate	0	14	8	4	0	26
Fair	0	6	17	3	1	27
Marked	0	3	1	13	1	18
Spongy	2	0	2	0	1	5
Totals	6	25	28	20	3	82

TABLE 4

	DATE STARTED	1 Slight	2 Moderate	3 Fair	4 Marked	5 Spongy	TOTAL	AVERAGE GRADE
Total stock	May 2	1	4	2	2	1	9	2.5
Total outcrossed	May 2	2	5	16	14	1	37	3.1
Total stock	May 5	4	31	28	10	3	73	2.6

Standard deviation of stock, 0.81 grades; of outcrossed, 0.84.

In the totals and averages the number of spongy winged flies were not included, because the position of this grade in the series was uncertain, but the number of these flies was negligible.

It will be seen that here, too, the rather unexpected result was obtained, that not only was there no evidence of contamination but the outcrossed was, if anything, less normal than the stock, and did not show a greater variability.

It should be mentioned that the same result had previously been obtained in the case of the character dachs legs, when comparisons were made between stock flies and outcrossed flies derived from the race $\frac{S\ d\ j\ c\ ba^2}{b\ p\ v\ a\ sp}$. In this case, however, only a few flies were examined, and those of the outcrossed race had not been freed from the other mutant factors, which were present in heterozygous condition.

[2] Muller, H. J. The mechanism of crossing-over. American Naturalist July, 1916.

CONCLUSION

The results above recorded give further evidence of the frequency with which characters are variable and change genetically without any artificial selection. In the case of the characters dealt with in this paper at least the variation that was observed was certainly not due to contamination or fractionation. This was true in spite of the fact that the factors had been kept in a state of heterozygosis for over fifty generations.

It should be noted that if the balloon and the other characters observed had had a higher survival value than the normal instead of a lower, they might have varied in the opposite direction from that observed and the results might then have been mistakenly attributed to contamination. This fact emphasizes the importance of not accepting results apparently showing contamination or fractionation of factors at their face value without a thorough factorial analysis.

THE RELATION BETWEEN SPECTRAL COLOR AND STIMULATION IN THE LOWER ORGANISMS[1]

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FOUR FIGURES

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[1] Contribution from the Nela Research Laboratory, National Lamp Works of the General Electric Company.

The literary work connected with this paper was done at the Johns Hopkins University; the experimental work at the Nela Research Laboratory, Cleveland, Ohio, during the summer of 1915 (July–September).

It is a genuine pleasure to express my appreciation of the courtesies extended on every hand by the director of this laboratory and his associates. Much expert advice of the greatest importance, especially that concerning the manipulation of electrical apparatus, was received from various members and the results obtained both in quantity and in quality are in no small measure directly related to the generous interest taken in my work and to the unexcelled facilities placed at my command.

INTRODUCTION

But little interest was manifested in the response of animals to colors until some time after the appearance of the Origin of Species (1849). This work at once greatly stimulated research in comparative morphology and embryology, with the result that there was soon collected a mass of material in support of the theory of evolution in so far as it pertains to the structure of organisms. In connection with this work on the origin of structure there continually arose problems concerning the evolution of functions, reactions, behavior, consciousness, etc. Among these was the question as to the relation between color and stimulation. This question was associated with the problem of evolution of psychic phenomena, and that of the evolution of color-patterns in animals and plants especially those in the flowers.

As applied to color-patterns in animals, the theory of evolution demanded color-vision on the part of the animals involved, for the patterns were considered to be associated with concealing phenomena and sexual selection; and as applied to flowers it demanded color-vision in those insects which have to do with pollination, for it was assumed that color is a determining factor in attracting insects and consequently that the reproduction of a plant depends upon the color of its flowers. But these ideas did not result in as much experimental work on the response of animals to colors as did the problem of the evolution of psychic phenomena.

It was at this time maintained that if psychic phenomena originated by the process of evolution, one ought to find evidence of mental faculties in the lower organisms. And there soon appeared a group of investigators who took up the study of the behavior of animals primarily in search of just such evidence. Prominent among these may be mentioned Bert ('69), Darwin ('80), Lubbock ('81), Romanes ('83), Graber ('83) and Preyer ('86). In general, the results obtained by the men just mentioned led them to conclude that psychic phenomena extend well down in the scale of animal life, if not to the very bottom.

Some of these investigators maintained that all classes of vertebrates, some of the arthropods and even some of the worms have color-vision. They used the so-called preference method in their investigations on the response of animals to colors, and largely owing to this and to the fact that they proceeded from a psychological point of view, their work has been severely criticised. However, a number of them, Lubbock in particular, used monochromatic light of high purity, and made a very thorough objective investigation of the subject; and moreover, a large proportion of their results has been abundantly confirmed. But many of their conclusions, especially those concerning subjective sensations have not been generally accepted.

Botanists have been interested in the response to colors very largely from a purely physiological point of view, and a number of them have made very extensive and thorough studies of the subject, using spectra of high purity, e.g., Guillemin ('58), Wiesner ('79), Strasburger ('78), and Blaauw ('09). In general it has been found that the region of maximum stimulation for green plants is in the blue, although some hold that it is in the violet; and that for fungi it is somewhat nearer the red end of the spectrum than it is for green plants. The most extreme shift in this direction that is known was discovered by Engelmann ('82) in his work on Bacterium photometricum in which he found a primary and a secondary maximum, the former in the infra-red and the latter in the orange.

A number of investigators have also been interested in the study of responses of animals to colors largely from the point of view mentioned above. Prominent among these may be mentioned Engelmann ('82), Verworn ('89), Hess ('10) and others.

Loeb should probably also be included in this group. His aim was, however, quite different from that of the other investigators mentioned. He was not interested in the evolution of consciousness, and he objected strenuously to the conclusion that psychic phenomena are involved in the reactions of animals. In support of this objection he attempted to prove that the reactions in plants and animals are fundamentally identical, maintaining that such a proof would show that there are no

psychic phenomena involved in the reactions of animals. One of the points of identity that he undertook to establish referred to the relation between color and stimulation in plants and animals. He rejected the results obtained by earlier investigators (Lubbock, Graber and others) which militated against his idea of identity, on the ground that the method employed by these investigators is faulty. They ascertained in which of two or more colors the organisms tend to aggregate, i.e., they used the so-called preference method. Loeb maintained that the results thus obtained have no bearing on the question as to the relation between color and orientation, the phenomenon primarily involved in his idea of identity. He consequently studied directly the effect of color on orientation.

His methods in the earlier work ('90) were, however, very crude. He used only two different colors, red and blue, and the constitution of neither was known, being produced by colored glass or colored solutions. Observations were made on the following animals: musca larvae, plant lice, caterpillars of Porthesia chrysorrhoea, moths of Sphinx euphorbia and Geometra piniaria, various copepods, the meal worm, Tenebrio molitor, and larvae of the June bug, Melolontha vulgaris, Limulus polyphemus and Polygordius. Loeb maintains that all of these animals responded in blue just as in white light and in red just as in darkness, and he concluded that in this respect the reactions in plants and animals are identical, in spite of the fact that Kraus ('76) had demonstrated that the stalks bearing the perithecal heads of the fungus, Claviceps microcephala, turn toward the light nearly as rapidly in the red as in the blue and Brefeld had obtained similar results for Pilobolus microsporus and Pilobolus crystallinus. In 1910 Loeb for the first time made use of spectral colors. He and Maxwell tested Daphnia, Balanus larvae and Chlamydomonas using the preference method, so severely criticised in his earlier work, and found the green or yellow region in the spectrum to be the most effective for all, thus confirming the results obtained on Daphnia by Bert ('69) and Lubbock ('81) which had been rejected in his earlier work.

Later Loeb and Wasteneys ('15–'16) found the region of maximum stimulating effect in a carbon-arc prismatic spectrum to be in the yellow (560–578 $\mu\mu$), for Balanus larvae; in the blue (460–480 $\mu\mu$), for Eudendrium; in the Blue near 495 $\mu\mu$ for Arenicola larvae; in the blue (460–490 $\mu\mu$), for Euglena viridis and in the green about 535 $\mu\mu$ for Chlamydomonas. These results are in full harmony with those of earlier workers in so far as they indicate that the relation between wave-length and stimulation is not the same for all animals. Loeb, however, still holds that it is the same for plants and animals, for he maintains that the fact, that the maximum in Chlamydomonas is in the green, shows that for plants it is not exclusively in the blue, just as has been found to be true for animals. This fact was, however, fairly well established before his first work appeared, as reference to the following table (table 1) containing a summary of the results obtained in previous experiments will show. Other conclusions reached by Loeb and Wasteneys will be considered later.

The more important conclusions that a study of this table warrants may be summarised as follows:

1. A large proportion of the investigations were made with the use of prismatic spectra, insuring fairly pure monochromatic light, but in some gaslight was used as a source of illumination, in others electric light and in still others sunlight. Moreover, in some experiments the effects of the different colors on the process of aggregations were studied, in others the effects on orientation and in still others the effects on activity. The distribution of energy in the different spectra used is however, similar in all and in all cases in which two or more of the three reactions mentioned were studied on the same organisms, the results were essentially the same, indicating that the relative stimulating effect of the different wave-lengths is the same for all three sorts of response. It is consequently possible to compare directly the results obtained by most of the different investigators as they are given in the table; but these results refer only to the relative stimulating effect of rather large regions in the spectrum and they are of such a nature that they show but little more than

TABLE 1

Summary of previous experiments on reaction to colors in plants and lower animals

Plants

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Poggioli	1817	Seedlings of Brassica and Raphanus	Spectrum	Turned toward light in red and more strongly in violet. Other colors not mentioned
Payer	1842	Seedlings	Solar prismatic spectrum and color media	Green, yellow, orange and red act like darkness. Turn toward light in violet and more strongly in blue
Gardner	1844	Seedlings	Solar prismatic spectrum	Seedlings turn toward light in all colors but most strongly in indigo
Dutrochet and Pouillet	1844	Roots of white and black mustard	Strong solar prismatic spectrum	All rays active including infra-red and ultra-violet but blue most active
Guillemin	1858	Seedlings of cress and Sinapis alba	Solar prismatic spectrum. 25 tests made	Primary maximum effect in violet or ultra-violet. Secondary between infra-red and green; minimum effect near boundary between blue and green (490 $\mu\mu$)
Sachs	1864	Seedlings	Red and blue solutions and glass	In red no reaction, in blue strong curvature
Müller	1872	Cress and Sinapis alba	Solar prismatic spectrum	Maximum for cress in blue at 490 $\mu\mu$. Maximum for Sinapis, green at about 550 $\mu\mu$. In weaker light maximum nearer red
Kraus	1876	Claviceps (fungus)	Colored media	Reaction in red nearly as strong as in blue
Brefeld	?	Pilobolus	Colored media	Reaction in red nearly as strong as in blue

TABLE I—Continued

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Wiesner	1879	Roots and plumules of seedlings	Solar spectrum and tested absorbing media	Maximum effect in violet. Secondary maximum in red. Maximum in green
Blaauw	1909	Seedlings of Avena Plycomyces	Spectrum. Equal energy	Maximum efficiency for Avena in blue 465 $\mu\mu$, for fungi nearer red
		Unicellular green forms		
Cohn	1865	Swarm spores	Colored glass	Red no response. Blue most effective on orientation
Strasburger	1878	Swarm spores, mainly Botrydium	Solar spectrum, quartz prism, colored glass and solutions; orientation	Violet, indigo and blue only rays cause orientation. Maximum in indigo. Other colors cause quivering movement
Engelmann	1882	Oscillaria	Solar and gas microspectrum	Active in red, orange and yellow, not in other colors
Engelmann	1882	Paramecium bursaria	Solar and gas microspectrum	Collect in red (650–700 $\mu\mu$). Probably reaction to oxygen
Engelmann	1882	Euglena viridis	Solar and gas microspectrum	Collect in blue (470–490 $\mu\mu$)
Engelmann	1882	Bacterium photometricum	Solar and gas microspectrum	Maximum collection in infra-red (800–900 $\mu\mu$). Secondary collection in orange (580–610 $\mu\mu$)
Verworn	1889	Oscillaria	Colored solutions and glass red, yellow, green, blue	Oscillaria orient in all colors, diatoms only in blue and violet
Loeb and Maxwell	1910	Chlamydomonas	Spectrum prismatic and normal	Maximum aggregation about 520 $\mu\mu$

TABLE 1—Continued

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Loeb and Maxwell	1910	Chlamydomonas	Carbon-arc spectrum	Maximum effect on orientation about 535 $\mu\mu$
Loeb and Maxwell	1910	Euglena viridis	Carbon-arc spectrum	Maximum aggregation between 460–510 $\mu\mu$ about at 485 $\mu\mu$. Maximum effect on orientation 460–490 $\mu\mu$
		Lower animals		
Bert	1869	Daphnia	Electric spectrum	Maximum aggregation in yellow and green. Orientation in all colors most active in yellow and green
Merejkowsky	1881	Dias and larvae of Balanus	(?)	In equal brightness, effect same in all colors
Lubbock	1881	Daphnia pulex	Solar prismatic spectrum	Maximum aggregation in green. Order of preference, G, Y, (*B*, *R*, *V*)
Wilson	1891	Hydra viridis	Colored glass and gas spectrum	Maximum aggregation, blue (430–490 $\mu\mu$)
Yerkes	1899	Simocephalus	Welsbach gas prismatic spectrum	Maximum aggregation yellow. Order of preference Y, O, G, R, B, V
Harrington and Leaming	1900	Ameba	Colored media and spectrum	Maximum effect on activity violet
Mast	1909	Ameba proteus	Solar prismatic spectrum	Maximum effect on activity blue (430–490 $\mu\mu$)
Loeb and Maxwell	1910	Daphnia	Prismatic and normal spectrum	Maximum aggregation in green
Loeb and Maxwell	1910	Balanus (larvae)	Prismatic and normal spectrum	Maximum aggregation in green
Hess	1910	Daphnia	Nernst glower prismatic spectrum	Movement of eye, order of effect beginning with maximum G, Y, B, R

TABLE 1—Continued

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Hess	1910	Porthesia chrysorroea (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Hyponomenta variabilis (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Dasychira fascelina (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Lasio campa potatona (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Phrogenatobia fulignosa (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Culex pipiens (larvae)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Culex pipiens (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Coccinella septempunctata (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow

TABLE 1—Continued

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Hess	1910	Bees (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Home and ichneumon flies (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Podapsis slabberi (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation; order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Atylus swammerdami (adults)	Nernst glower prismatic spectrum	Rate of movement or region of aggregation: order of effect G, Y, B, R. Maximum in green near yellow
Hess	1910	Mussels (several species)	Nernst glower prismatic spectrum	Movement of siphon (Y-G), B, O, R
Hess	1910	Cephalopods	Nernst glower prismatic spectrum	Reaction of pupil (Y-G), B, V, R
Hess	1910	Amphioxus	Nernst glower prismatic spectrum	Maximum activity in (Y-G)
Day	1911	Crayfish	Spectral colors equal in energy B-V = 430–490 $\mu\mu$; Y-G = 524–576 $\mu\mu$; R = 625–665 $\mu\mu$	Pigment migration in eye about equal in (B-V) and (Y-G). Much less in R
Gross	1913	Calliphora erythrocephala (larvae)	Four spectral colors equal in energy*	Order of efficiency beginning with maximum: G, B, Y, R

TABLE 1—Continued

INVESTIGATOR	DATE	ORGANISM	METHODS	RESULTS
Gross	1913	Zeuzera pyrina (larvae)	Four spectral colors equal in energy*	G, B, Y, R
Gross	1913	Feltia subgothica (larvae)	Four spectral colors equal in energy*	G, B, Y, R
Gross	1913	Feltia subgothica (adults)	Four spectral colors equal in energy*	B, G, Y, R
Gross	1913	Calliphora erythrocephala (adults)	Four spectral colors equal in energy*	B, G, Y, R
Gross	1913	Drosophila ampelophila (adults)	Four spectral colors equal in energy*	B, G, Y, R
Gross	1913	Periplaneta americana (adults)	Four spectral colors equal in energy*	Equally strongly negative in G and Y. Positive in B, no response in R
Frisch and Kupelwieser	1913	Daphnia	Colored media	Positive orientation in R, Y, G. Negative orientation in (B–G), B, V
Ewald	1914	Daphnia	Colored media and spectrum	Maximum effect on positive orientation in red (650–660 $\mu\mu$); on orientation (410–420 $\mu\mu$)
Loeb and Wasteneys	1916	Eudendrium	Carbon-arc spectrum	Maximum effect on orientation in blue (460–480 $\mu\mu$)
Loeb and Wasteneys	1916	Balanus (larvae)	Carbon-arc spectrum	In yellow (560–578 $\mu\mu$)
Loeb and Wasteneys	1916	Arenicola (larvae)	Carbon-arc spectrum	In green about 495 $\mu\mu$

*Blue (420–480 $\mu\mu$), Green (490–550 $\mu\mu$), Yellow (570–620 $\mu\mu$), Red (630–655 $\mu\mu$).

which of these large regions is most effective. Concerning relative efficiency all that can be ascertained is that all waves longer than those in the region of maximum effect are less efficient than those in this region, for they contain relatively more energy. Regarding the efficiency of the shorter waves which contain less energy than those in the region of maximum effect no definite statement can be made.

Only three of the investigators, Blaauw, Day and Gross, attempted to ascertain the relative stimulating efficiency of the different wave-lengths. Day and Gross used spectral colors equal in energy but they tested only four different colors. Blaauw tested more colors but I am not certain as to the method used, having access only to an abstract of his paper.

2. For seedlings of green plants, plumules and radicles, the region in the spectrum of maximum stimulating effect is in the blue or violet. For the fungi it is somewhat nearer the red. For Bacterium photometricum it is in the infra-red and the orange. For Oscillaria and Paramecium bursaria is it questionable, activity and aggregation being probably determined by chemical changes in the solution associated with the colors. For Chlamydomonas it is in the green; for all other unicellular forms tested it is in the blue, as it is also for the cœlenterates and vermes and for a few of the molluscs and arthropods. But for most of the molluscs and arthropods it appears to be in the green or yellow.

In none of the organisms mentioned in the table are the reactions specifically associated with the wave-lengths; they are not entirely independent of intensity. If, e.g., the green were made relatively sufficiently intense, the region of maximum effect could be changed from the blue to the green, etc. In the bees, however, and in many of the vertebrates, the evidence obtained indicates that the reactions may be independent of the relative intensity. Bees, e.g., can be trained to select any given color, regardless of its intensity in relation to that of other colors; and such reactions to colors are the only ones which are like the reactions associated with color-vision in man.

I have for some time held the opinion that the study of reactions to colors, aside from its importance in comparative psychology and physiology, ought to yield results which will throw light on the nature of those chemical changes in the organisms, which are associated with the reactions to light (Mast '11, pp. 320, 363). But for this purpose it is necessary to ascertain more in detail the relation between the wave-length and stimulation than it has been ascertained in previous work. It is highly essential to use monochromatic light of such a nature that it can be measured directly or indirectly in terms suitable for comparative work, preferably in terms of energy, so as to be able to give the relative stimulating efficiency of the different wave-lengths. It is also highly essential to ascertain the stimulating efficiency for all regions in the spectrum that are at all effective, not merely for those that are most effective. These ideas have been the guiding principles in the following experiments.

METHODS AND MATERIAL

The methods used in the following experiments are based upon those used in observations on orientation of organisms in a field of light consisting of two beams crossing at right angles ('07, pp. 132–134; '11, pp. 86–89). In these observations it was found that among the organisms which orient all of those without image forming eyes proceed toward or from a point situated between the two beams and that the location of this point depends upon the relative effectiveness of these two beams. If the illumination in the two beams is the same in quality and quantity, so that the stimulating effect is the same, then the point lies half way between them, provided the organisms in a single beam travel parallel with the rays and do not deflect to the right or the left.

This being true, it is obvious that whenever such organisms, exposed to light from two sources, proceed toward or from a point midway between them it may be concluded that the light received from the two sources is equal in stimulating effect, no matter how much it may differ either in quantity or in quality.

Consequently, it is evident that we have here a method by means of which the stimulating effect of light differing in wave-length can be compared and the relative stimulating effect ascertained. And if this is known it is a simple matter to calculate the relative stimulating efficiency provided the relative energy of the different wave-lengths compared is known.

The simplest method of procedure is to keep the quality of the light (white, e.g.) in one beam constant while that in the other is changed and then to adjust the illumination from the white light in the one beam for each change of wave-length in the other until, in each case, the organisms proceed along the same path. The stimulating effect of the different wave-lengths tested will then be directly proportional to the various illuminations from the white light required to make the organisms, under each of the different conditions, proceed in the same direction. For example, if for the green it requires twice as much light from the white source to make the creatures take a given course as it does for the yellow, then the stimulating effect of the green is twice as great as that of the yellow, and if the yellow has twice as much energy as the green then the stimulating efficiency of the green is four times as great as that of the yellow. This method is applicable to organisms which, in light from a single source, do not tend to travel parallel with the rays, but tend to deflect to the right or the left, e.g., Volvox, as well as to those which do not tend to deflect, provided the extent of the deflection is equal under the various conditions.

In the experiments described in the following pages, two 80-watt, gas-filled, street series, tungsten lamps with vertical coiled filaments were connected in series with a 40-volt storage battery and enclosed in light-proof boxes each containing an opening of such a size and so situated as to produce a small horizontal beam of light. One of these beams passed through a Hilger constant deviation spectrometer and a lens which focused the filament on the slit, the other passed through a Lummer-Brodhun rotating sector. The whole apparatus was so arranged that the two beams of light crossed at right angles in the field of observation as represented in figure 1.

By means of the higher spectrometer the organisms could be subjected in the one beam to any spectral color and the change from one to another could be rapidly made without in any way altering the size or position of the beam of light. Moreover, the wave-length of the light in the beam could be readily ascertained by direct readings on the revolving drum of the spectrometer.

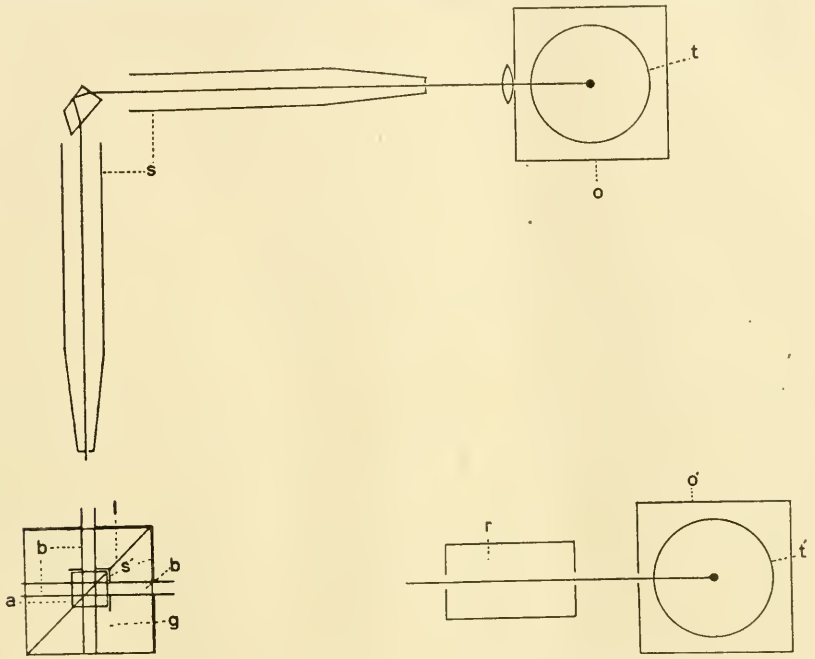


Fig. 1 Outline of apparatus used in the following experiments: t and t', tungsten street series lamps; o and o', opaque boxes; s, Hilger spectrometer; r, Lummer-Brodhun rotating sector; b, beams of light, 10 mm. wide, 3 mm. deep; s', opaque screens; g, glass plate; l, line on glass plate; a, aquarium.

By means of the Lummer-Brodhun sector the illumination in the other beam could be instantly changed from zero to the maximum available and the illumination used at any time could be directly ascertained by readings on the sector.

All of these features and the constant current from the storage battery were found to be of the greatest importance in the work undertaken. In fact, it would have been quite impossible to have obtained some of the most important results without them.

The two slits in the spectrometer were both, unless otherwise stated, 0.75 mm. wide, insuring a fair degree of purity in the spectral colors used. All of the observations on the microscopic forms were made in a small rectangular aquarium under a binocular supported by an extension arm. The aquarium, which was 26 mm. wide, 26 mm. long and 9 mm. deep, was made of the best quality of colorless glass slides accurately cut and ground and glued with Khotinsky cement. An opaque screen containing an opening 3 x 10 mm. was fastened to each of the two adjoining sides of the aquarium in such a position that the long axis of the opening was parallel with the bottom, the lower edge of it just a trifle lower than the upper surface of the bottom and the ends of it equal distance from the ends of the aquarium. Thus each of the two horizontal beams of light crossing each other at right angles in the aquarium was 10 mm. wide. The aquarium was supported by a glass plate containing ledges so arranged that the aquarium could be easily removed and returned to precisely the same position. The glass plate contained a line which passed under the center of the aquarium and bisected the angle between the two beams of light. Some distance under the center of the glass plate there was a 2 c. p. ruby electric bulb and above this a plate of ground glass and a petri dish containing water for the purpose of diffusing the light and reducing the heat (fig. 2). In some of the work the dish was replaced by a few additional glass plates. The light from below made it possible to see the organisms and the line on the glass plate clearly, but being red it did not appreciably influence their direction of movement. For further information regarding apparatus used see page 517.

In making the observations, a considerable number of the organisms was put into the aquarium in clear pond or tap water about 4 mm. deep. The aquarium was then put in place and the illumination in the beam of white light adjusted by altering the size of the opening in the Lummer-Brodhun sector until the organisms travelled parallel with the line under the aquarium on the glass plate. This adjustment was made in darkness, so as to eliminate the personal factor as a possible error. After the

reading indicating the extent of the opening of the sector was recorded, the wave-length was changed, the water in the aquarium thoroughly stirred up so as to distribute the organisms equally throughout unless otherwise stated, and the illumination in the white beam again adjusted until they proceeded parallel with the line under the aquarium. This was repeated for all effective regions of the spectrum usually differing by 10 $\mu\mu$. Thus the readings indicating the extent of the opening of the sec-

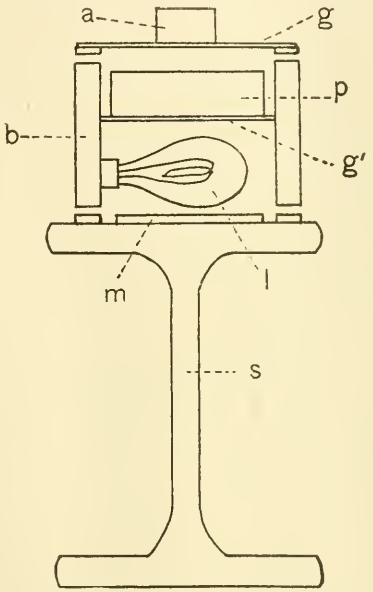


Fig. 2 Side view of observation aquarium, stand, etc. *a*, aquarium; *s*, stand; *b*, opaque box, 8 x 10.7 x 10.7 cm.; *l*, ruby light; *m*, mirror; *g*, glass plate; *g'*, ground glass plate; *p*, petri dish. The binocular was supported by an extension arm vertically over the aquarium.

tor, when the course of the organisms bisected the angle between the two beams of light, were ascertained for the different regions; and since these readings directly express the relative illumination, they express also, as previously demonstrated, the relative stimulating effect of the regions tested; and from these data the relative stimulating efficiency of the different wavelengths with reference to the energy can readily be calculated provided the relative distribution of energy in the spectrum

used is known.[2] It was consequently not necessary to make measurements of the absolute illumination used, and this constitutes one of the most advantageous features of the method employed, for it simplifies greatly the work involved. We shall, however, give sufficient data in the following paragraphs and in connection with the description of the individual experiments to make it possible to reduce the readings as presented in the following pages to terms of absolute intensity in case anyone should desire to do so.

In all but a few preliminary experiments, which were made before August 1 the current used was 5.75 amperes with only very slight variation. With this current and with the Lummer-Brodhun sector set at 100, the candle power in the white beam was 40.6. In all of the experiments except those on earthworms and blowfly larvae the source of light in the white beam was 50 cm. from the center of the observation aquarium. The illumination in this beam with the sector set at 100 was therefore approximately 162.4 meter-candles at the center of the field of observation. It was actually somewhat less owing to reflection and absorption. To calculate the illumination with the sector set at any other point it is only necessary to read the number on the scale opposite the point in question, divide it by 100 and multiply the quotient by 162.4, i.e., by the illumination in meter-candles with the sector at 100. In this way the illumination can readily be calculated for any of the sector readings in the following tables except those which refer to earthworms and blowfly larvae. In the experiments on these two forms the distance between the center of the field of observation and the source of light in the white beam was 100 cm. and the light was also reduced by screens so that with the sector set at 100 the candle-power was in some of the experiments 21.4, in others only 7.4. These numbers must therefore be used in place of the preceding in calculating the illumination. In these experiments the distance between the ocular slit of the spectrometer and the center of the field of observation was 53 cm. and both beams of light were 6 cm. wide (see p. 517).

[2] See figures 3 and 4, and tables 2 to 15.

The relative distribution of energy in the spectrum used in the following experiments was ascertained by Dr. W. E. Forsythe and Mr. Francis E. Cady, members of the staff of the Nela Research Laboratory. Mr. Cady also measured the light used. I am greatly indebted to these gentlemen for their generous assistance.

At the end of the experiments the lamp used was matched in color with one of the laboratory standard lamps whose distribution was known in terms of a standard radiator. A new lamp precisely like the one used in the experiment was then similarly matched and the results agreed so closely that it was evident that there was no appreciable change in the lamp during the time it was used in my experiments and that the distribution of energy in the spectrum remained practically constant. The results of the above mentioned tests are plotted in figure 3.

By referring to the accompanying curve it will be seen that beginning at the violet end of the spectrum and proceeding toward the red end the energy first increased rather gradually but later very rapidly, so that while there was, in the green at 560 $\mu\mu$, $30\frac{2}{3}$ times as much energy as in the violet at 400 $\mu\mu$, there was, in the red at 700 $\mu\mu$, more than 138 times as much. This great difference in energy in different regions of the spectrum shows clearly that if one should obtain a response in the longer waves and none in the shorter, it would not necessarily prove that the stimulating efficiency of the former is greater than that of the latter, for the difference in the response might be due solely to the difference in the amount of energy involved.

To ascertain the relation between wave-length and stimulating efficiency, it is consequently necessary to make corrections for the unequal distribution of energy in the spectrum. Such corrections were made in all of the experiments described in the following pages.

Most of the organisms investigated were collected in temporary clay pools formed in the immediate vicinity of the laboratory at Nela Park, owing to the unprecedented abundance of rainfall during the season. In these pools, many of which were so small that they contained only a few gallons of water, unicellular and

colonial organisms appeared in great abundance, especially the green forms, Chlamydomonas, Euglena, Pandorina and the like. In any given pool a given species ordinarily predominated at any given time, but usually there was a succession of species between successive rain storms, depending apparently upon changes in the

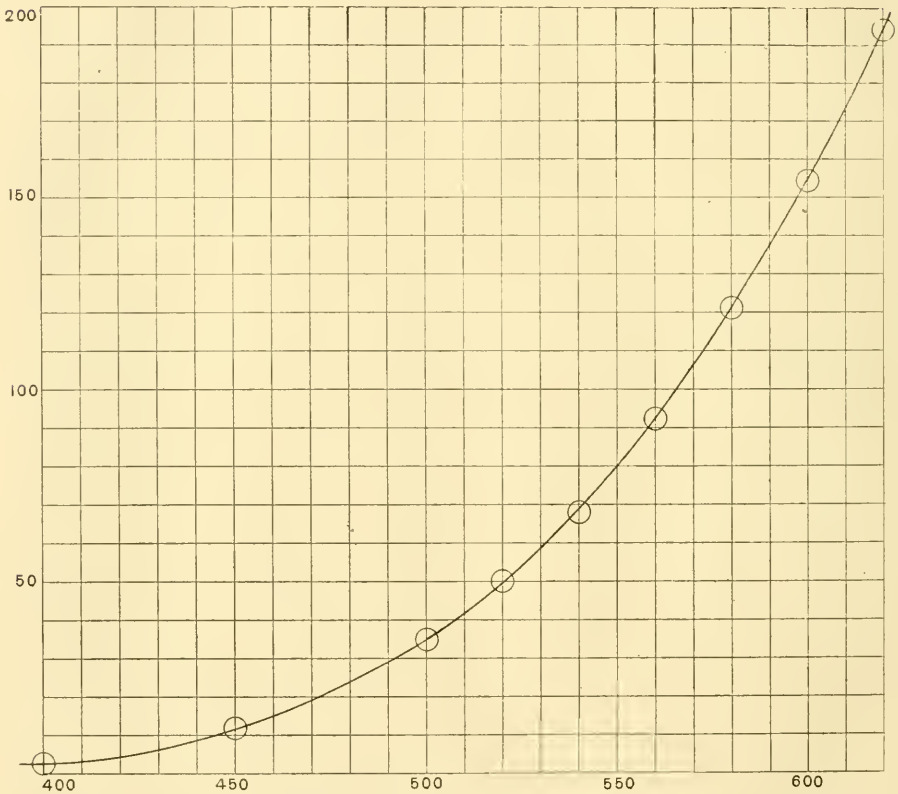


Fig. 3 Curve representing the distribution of energy in the spectrum used in the following experiments. The circles represent the points experimentally established, the ordinates the energy and the abscissae the wave-lengths in $\mu\mu$.

constitution of the solution. For example, in one particular pool which frequently dried up entirely Chlamydomonas appeared first each time after several rain-storms and became very abundant with a few scattered Pandorina colonies. Then after a few

days Pandorina became very abundant while the other organisms practically disappeared. A few days later Eudorina developed, while Pandorina gradually disappeared. Thus by watching these pools many of the species studied were secured in any desired numbers and practically pure. Among these were several of which ordinarily only a few scattered individuals are found, e.g., Euglena tripteris, Trachelomonas, Eudorina, and Gonium. This fortunate location of an abundance of excellent material close at hand greatly facilitated the work undertaken. In fact it would otherwise have been quite impossible to have so thoroughly covered such a large field in the few months at my disposal. The following fifteen species were fairly thoroughly investigated with reference to responses to colors, and a number of others were superficially studied. Trachelomonas, Chlamydomonas, and Phacus each one species; Euglena five species, Gonium, Pandorina, Eudorina, Spondylomorum, Lumbricus, Arenicola (larvae) and blowfly (larvae) each one species. Details concerning these studies and the results obtained are presented in the following pages.

EXPERIMENTAL OBSERVATIONS

Introduction

Success or failure in attempting to ascertain the relation between wave-length and stimulation with the method previously described depends largely upon the condition of the organisms used. The more precisely they orient, the more strongly positive or negative and the more active they are, the more accurate the results will be. It is consequently essential, especially for those who may wish to repeat these experiments, to know as much as possible about the treatment the organisms received preceding the tests, their habits and habitats and the conditions under which they are likely to respond in a way most favorable to the work. I shall therefore in this section present, in addition to the results obtained, some of the characteristics of the responses of the organisms, their habits, and their environment which are intimately related to the observations made.

Euglena viridis

The specimens of Euglena viridis used in the following experiments were collected in a pig-yard in Michigan, near Ann Arbor, August 17, 8 a.m., taken to Cleveland and placed in a jar in

TABLE 2

Euglena viridis (negative). Relation between wave-length and stimulation[1]

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS							RELATIVE STIMULATING EFFICIENCY CALCULATED ON BASIS OF EQUAL ENERGY
	Results of individual tests						Average	
422.4[2]					1.5		1.5	2.50
432.6		2.5			4.0	3.2	3.233	4.09
442.8		9.0	12.5		8.2	12.8	10.625	10.41
452.9		17.0	18.5		15.7	18.2	17.350	13.24
463.1	27.5	19.4	23.0	23.5	24.0	32.5	24.983	14.86
473.2	38.5	36.0	40.5	34.8	38.9	38.7	37.900	18.22
483.4	53.5	50.9	55.0	46.3	46.7	61.0	52.233	20.40
493.6	41.2	35.5	51.0	47.3	42.1	51.9	44.833	14.46
503.7	23.3	27.3	30.2	32.4	34.6	35.5	30.550	8.23
513.8		11.6	14.9	9.9	16.7	14.6	13.540	3.06
524.0		7.0	5.0	?		5.0	5.666	1.08
Time	August 18 5.10–6.02 p.m.			August 19 10.43–11.37 a.m.				

[1] The figures in the columns 2–7 express relatively the stimulating effect of the different wave-lengths tested. The larger the number the greater the effect. Thus in column 3 the numbers show that the effect of wave-length 432.6 $\mu\mu$ was very slight and that from this point toward the red end of the spectrum the effect gradually increased until it reached a maximum near 483.4 $\mu\mu$, after which it gradually decreased until it became practically zero at 524 $\mu\mu$. The blank space in the columns indicate either that no readings were made or that the stimulating effect was so small that it could not be successfully measured by the method employed (see pp. 483–487).

The numbers in the last column indicate the distribution of stimulating effect in a spectrum having a uniform distribution of energy throughout, i.e., they express the relative stimulating efficiency of the different wave-lengths. These numbers were obtained from those in the preceding column by applying the corrections for unequal distribution of energy indicated in the curve in figure 3.

[2] The readings were presumably all made 10 $\mu\mu$ apart as the drum of the spectrometer indicated, i.e., at 420, 430, 440 $\mu\mu$, etc. It was, however, discovered by Dr. W. E. Forsythe that this series of readings on the drum, owing to imperfect adjustment, produced the wave-lengths given in the first column of this and the following tables.

rather weak diffuse daylight. The following day some specimens, together with about 2 cc. of clear solution, were taken from this jar, put into the observation aquarium and tested in the spectrum. They were found to orient fairly accurately and to be strongly negative. A series of tests covering different regions of the spectrum was then made as described in the preceding section. The results obtained in these tests are given in table 2, columns 2–4.

The following day, August 19, 9 a.m., specimens from the culture jar were tested in nearly pure, clear tap-water as well as in water from the jar. They were negative in both. The tests with the organisms in tap-water were now continued in the spectrum. Toward the close of the series of readings many of the specimens were positive but all of the observations were made on negative specimens. The results obtained in this series of tests may be found in columns 5–7, table 2. By comparing these results with those obtained in the preceding series of tests, it will be seen that they are essentially the same, showing that the impurities in the culture medium, which in most cultures give it a slightly brownish tint, do not appreciably affect the results. All of the following tests, unless otherwise stated, were made in clear solutions taken from the culture jars.

On August 20 in the afternoon specimens taken from the culture jar were found to be strongly positive, but toward the close of a series of tests made many of the specimens in the observation aquarium were negative. The results of this series of tests are given in table 3, columns 2 and 3. During the remainder of the day, specimens were taken from the jar at several different times and tested. They were found to be negative every time, but orientation was not precise enough to obtain accurate readings. The next day, August 21, the euglenae in the jar were again positive and in the afternoon orientation was fairly precise. The results of a series of tests made at this time are presented in columns 4 and 5, and those of another series made with positive specimens, August 23, in columns 6 and 7, table 3. The averages for all of the negative readings and those for all of the positive readings may also be found in these tables

as well as the results obtained in correcting the averages for unequal distribution of energy in the spectrum. The corrected averages are also plotted in figure 4.

TABLE 3

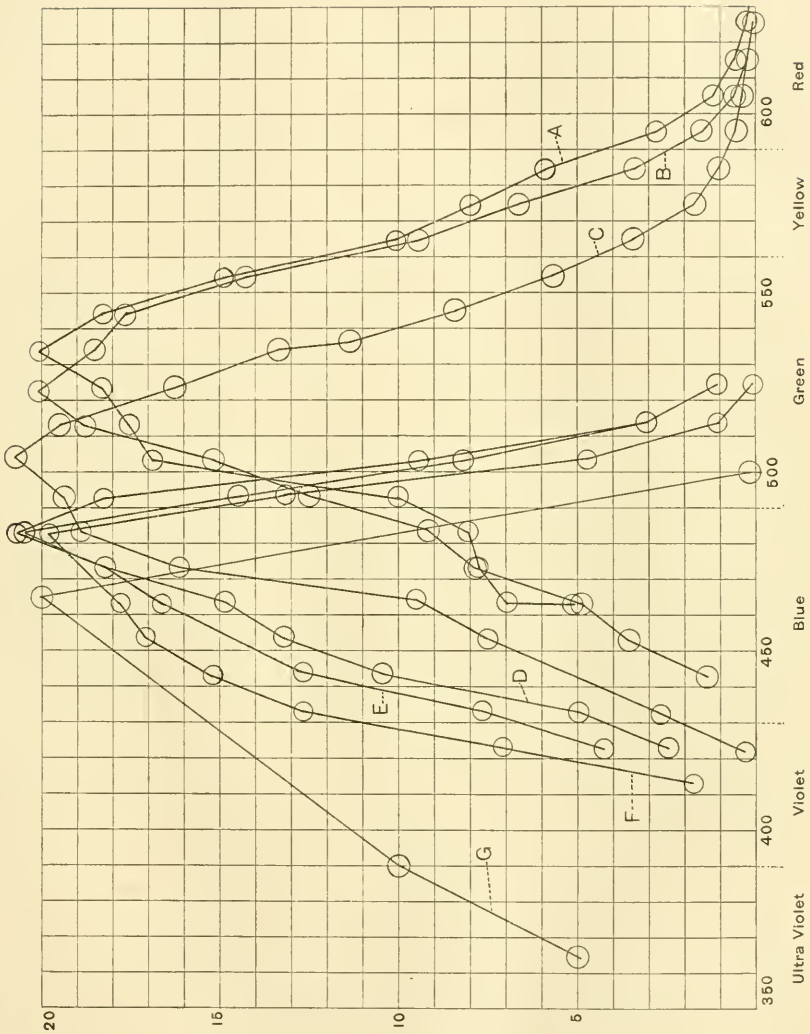
Euglena viridis (positive). Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS							RELATIVE STIMULATING EFFICIENCY CALCULATED ON BASIS OF EQUAL ENERGY
	Results of individual tests						Average	
432.6	1.7			2.3			2.000	2.53
442.8	4.9	7.7	3.7	5.0	4.9		5.240	5.13
452.9	14.0	14.7	7.8	8.7	10.0	11.4	11.100	8.47
463.1	22.0	24.7	14.0	15.0	18.8	17.5	18.666	11.11
473.2	30.0	32.6	22.7	20.9	23.1	23.0	25.383	12.20
483.4	39.4	45.0	32.1	31.3	30.5	35.4	35.616	13.91
493.6	44.9	40.7	36.4	34.7	35.6	34.8	37.850	12.20
503.7	34.4	25.3	19.0	16.6	22.4	22.8	23.416	6.31
513.8	14.9	11.5	7.5	5.8	8.5	7.3	9.250	2.09
524.0		3.8		0	3.3		3.550	0.67
Time	August 20 2.40–3.10 p.m.		August 21 2.25–2.52 p.m.		August 23 2.17–2.37 p.m.			

By referring to the two tables mentioned, to table 15, p. 521, and to figure 4 it will be seen that, while the readings for individual tests are considerably higher for the negative specimens than they are for the positive ones, due to changes in illumination or to deflection of the path of the organism, discussed elsewhere, the distribution in the spectrum of the stimulating effect is essentially the same for both. The region of maximum stimulation clearly lies between wave-lengths 483 and 493 $\mu\mu$ in both,

Fig. 4 Curves representing the distribution in the spectrum of stimulating efficiency, constructed from data given in table 15. *A*, Pandorina (negative); *B*, Pandorina (positive); *C*, blowfly larvae; *D*, Euglena viridis (negative); *E*, Euglena viridis (positive); *F*, Euglena tripteris (negative); *G*, Avena sativa (oats seedlings), constructed from data obtained by Blaauw. The circles represent points experimentally established; abscissae, wave-lengths; ordinates, relative stimulating efficiency on the basis of equal energy.

The curves for Eudorina and Spondylomorum, not represented in the figure, are in position and form essentially like those for Pandorina; the curve for Chlamydomonas is much like that for blowfly larvae; those for E. gracilis, E. minima, E. granulata, Phacus, Trachelomonas, Gonium, Arenicola, and Lumbricus are nearly like those for E. viridis and E. tripteris (see table 15).


20
15
10
5
350
400
450
500
550
600
Ultra Violet
Violet
Blue
Green
Yellow
Red
A
B
C
D
E
F
G

although it appears to be somewhat nearer 493 $\mu\mu$ for the positive than it is for the negative specimens. From this region the stimulating effect decreases in either direction at nearly the same rate for both. The distribution of stimulating efficiency is also essentially the same for both and much like the distribution of the stimulating effect. The maximum efficiency is, however, somewhat nearer the violet, being near wave-length 483 $\mu\mu$, and the efficiency of the shorter waves in general is relatively higher than the effect.

Loeb and Wasteneys ('16) in an interesting series of experiments located the region of maximum stimulating effect for Euglena viridis in a "carbon arc spectrum" between wave-lengths 460 and 490 $\mu\mu$. This region includes the much narrower region which we have established for this form, but the results obtained by Engelmann ('82) in a very thorough study of the behavior of the same species are considerably more nearly in harmony with ours.

Engelmann maintains (p. 398) that in a prismatic micro-spectrum thrown vertically upon a hanging drop under a microscope with the spectrometer slit 0.42 mm. or less wide, a large majority of the organisms collects in a narrow band in the blue between wave-lengths 470 and 490 $\mu\mu$ and that this result is the same with strong gaslight (Sugg's Brenner) as it is with daylight used as a source of illumination. With the spectrum divided into six fields of equal width he gives the results obtained in one experiment as follows and maintains that they were essentially the same in all of many other experiments performed:

Feld 1 (A bis $C\frac{3}{4}$ D): 2 Individuen.
Feld 2 ($C\frac{3}{4}$ D–$D\frac{5}{6}$ E): 0 Individuen.
Feld 3 ($D\frac{5}{6}$ E–$E\frac{5}{6}$ F): 16 Individuen.
Feld 4 ($E\frac{5}{6}$F–$F\frac{4}{7}$ G): 100 Individuen.
Feld 5 ($F\frac{4}{7}$ G–G): 24 Individuen.
Feld 6 (G–$G\frac{1}{2}$ H): 3 Individuen.

The individuals in fields 3 and 5, Engelmann says, were close to the edges of field 4, nearly all of them between wave-lengths 490 to 500 and 470 to 460 $\mu\mu$ respectively. According to this statement, field 4 was located between 470 and 490 $\mu\mu$. This is,

however, not fully in harmony with the limits of this field as given above, which according to my calculations indicates that it lies between 494.7 and 451.5 $\mu\mu$.

The aggregation of the euglenae under the conditions of the experiments was undoubtedly dependent upon shock reactions, for Engelmann says (p. 398): "Hier, zwischen den Wellenlängen 0.47 and 0.49 μ, fahren sie fort, sich in normaler Weise zu bewegen, kehren aber immer an der Grenze von Dunkel, von Grün, die meisten auch an der Grenze von Indigo oder Violett wieder um." Engelmann maintains moreover (p. 399) that by means of a microspectral objective he was able to ascertain the sensitivity of Euglena to changes of intensity in different regions of the spectrum and that he found "dass der kleinste für Euglena merkbare Intensitätsunterschied für Roth, Gelb, und Grün ansehnlich grösser ist als für Blau (zwischen 0.47 und 0.49 μ Wellenlänge) und auch für Violett.

The results obtained by Engelmann regarding the location of the region of maximum stimulating effect in the prismatic spectrum are in close agreement with those obtained by the writer and presented in the preceding pages. In the experiments of the former, however, the results depended upon shock-reactions (time rate of change of intensity) and in the latter upon orientation.

If the contention of Loeb and Wasteneys ('16, p. 235) is correct, that the photochemical substances in all organisms having the regions of maximum sensitiveness in the same location in the spectrum belong to the same type, then the photochemical substances associated with shock-reactions and those associated with orientation must belong to the same type. And if this is true it lends strong support to the contention of the writer and others that shock-reactions and orientation in Euglena are fundamentally the same, in fact, that orientation is the result of shock-reactions; and it militates against the contention of Loeb ('06), Bancroft ('13) and Torrey ('13) that these phenomena are fundamentally distinct.

Euglena gracilis

Euglena gracilis was found in great abundance in a small temporary clay pool at Nela Park, August 5 in the morning. Some

TABLE 4

Euglena gracilis (negative and positive). Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS							RELATIVE STIMULATING EFFICIENCY CALCULATED ON BASIS OF EQUAL ENERGY	
	Results of individual tests					Average			
	− Individuals			+ Individuals		− Ind.	+ Ind.	− Ind.	+ Ind.
432.6				0.7	2.1		1.4		1.77
442.8	3.2	5.0		5.5	8.5	4.1	7.0	4.02	6.86
452.9	10.1	10.0		13.8	13.2	10.05	13.5	7.67	10.30
463.1	19.0	17.3	15.5	17.6	20.0	17.26	18.8	10.27	11.19
473.2	21.8	27.6	29.0	26.1	26.2	26.13	26.15	12.56	12.57
483.4	27.7	36.1	33.0	32.4	33.6	32.26	33.0	12.60	12.89
493.6	21.1	24.1	24.7	26.6	27.0	23.30	26.8	7.51	8.34
503.7	9.3	14.0	7.3	11.2	11.2	10.20	11.2	2.74	3.01
513.8		1.5(?)		3.0	3.0	1.5 (?)	3.0	0.34(?)	0.67
524.0				2.8	0.7		1.75		0.33
Time	Aug. 5, 9.22–9.50 a.m.			Aug. 7, 4.24–4.59 p.m.					

For description of table see legend table 2, p. 492.

specimens were taken directly to the laboratory and tested. They were found to be strongly negative and to orient very precisely.

The results obtained in a series of tests made at this time may be found in table 4, columns 2 to 4. The figures in the second column represent the results of successive tests beginning at the bottom; those in the other two columns the results of tests made without any regular order of succession. All of these tests were made with the same individuals continuously in the observation aquarium. Toward the close of the experiments many of the individuals became positive, first in the lower illuminations and later also in the higher, and orientation became much less precise, making it impossible to obtain very accurate readings. About an hour later practically all of these individuals were positive and orientation was very precise. A series of tests was made at this time (11.23 to 11.46 a.m.), after which the specimens used were placed in weak light.

In the afternoon they were again quite as strongly negative as they had been when first brought in and another series of tests

was made (3.39 to 4.02 p.m.). The culture jar was now again placed in weak light and left two days, when specimens taken from it were found to be very strongly positive. The results of a series of tests made at this time are given in table 4, columns 5 to 6. The results recorded in the first of these two columns were obtained from tests made in successive order beginning in the green at 534.1 $\mu\mu$ and proceeding toward the blue and those in the second column from tests beginning in the violet at 422.4 $\mu\mu$ and proceeding in the opposite direction.

By comparing these two columns it will be seen that there is a remarkably close agreement between the two series of results obtained, although the two readings in each region of the spectrum were in no case made successively, and usually there intervened a considerable period of time between them.

These results illustrate the possibilities of the method employed, but results of such accuracy can be expected only when the organisms are in just the proper condition. The results obtained in two series of tests, one for negative and one for positive individuals, not recorded in the table are essentially the same as those recorded, although they are not quite so consistent and accurate. All of these results indicate clearly that the distribution of stimulating efficiency in the spectrum for Euglena gracilis is practically the same as it is for Euglena viridis (table 15, p. 521). The region for maximum efficiency is between 473.2 and 483.4 $\mu\mu$ near the latter, and from this region the efficiency decreases rapidly toward the red end of the spectrum and more slowly toward the violet end. Moreover, the stimulating efficiency for negative individuals appears to be the same as it is for positive ones in both species.

Euglena tripteris

One usually finds Euglena tripteris much scattered, a few specimens here and a few there. At Nela Park I was very fortunate in obtaining for a few days, from a little water which had collected in a wagon track on a clay road, an abundant supply of these creatures almost pure.

They were tested in the spectrum from time to time during four days (August 20 to 24) and were found to be almost con-

TABLE 5

Euglena tripteris (negative). Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS											RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY
	Results of individual tests										Average	
422.4		0.5(?)		0.8(?)							0.65	1.08
432.6	3.6	5.2	3.8	3.6	2.6						3.76	4.75
442.8	9.6	10.5	9.9	6.0	6.9						8.58	8.41
452.9	20.2	17.3	16.8	10.0	11.4	9.2			9.0		13.4	10.23
463.1	24.5	24.6	30.1	14.4	18.9	13.0	15.0		17.5	15.0	19.22	11.44
473.2	34.2	31.5	35.5	21.5	22.8	20.7	20.5	19.4	22.2	19.9	24.82	11.93
483.4	40.3	46.3	53.0	25.3	29.1	29.0	27.9	27.9	32.5	25.7	33.7	13.16
493.6	35.9	41.4	40.7	19.5	19.2	24.3	20.5	23.7	27.3	20.6	27.31	8.80
503.7	15.2	16.4	15.2		8.5	11.0	10.8		8.9	8.3	11.78	3.17
513.8	3.5		3.3		1.7	4.4			2.6		3.1	0.70
524.0					0.4	0.7					0.55	0.10
Time	Aug. 20, 10.50–11.40 a.m.			Aug. 20, 3.13–3.36 p.m.			Aug. 23,[1] 3.44–4.32 p.m.		Aug. 24, 11.30–11.48 a.m.			

[1] Kept in darkness 3.53 to 4.21 p.m.

For description of table see legend table 2, p. 492.

tinuously negative in all illuminations in which they responded at all. The results obtained in these tests are presented in table 5 and figure 4. In every case all of the individual tests represented in a given column were completed before any represented in any other column were made, and usually they were made in successive order up or down the columns.

By referring to table 5 it will be seen that the distribution of stimulating effect in the spectrum was essentially the same in all of the four separate groups of tests made and that the distribution of stimulating efficiency in this species is practically the same as it is in viridis and gracilis (table 15). But it will also be seen that while the sector readings made in the different groups of tests lead to the same conclusion regarding the distribution of stimulating effect in the spectrum they differ greatly in magnitude. For example, the sector readings at the maximum in the first group of tests were nearly twice as large as those in the second group, although in both the maximum was near

483.4 $\mu\mu$. This variation in magnitude was no doubt due to slight changes in the position of the image of the filament in the lamp on the slit of the spectrometer, caused largely by the bending of the filament in the process of heating. This contention is supported by the fact that inconsistent readings in a given group of tests were obtained only if during the process of making the tests in the group the lamp was turned off and allowed to cool and then turned on again.

TABLE 6

Phacus triqueter (negative). Euglena granulata (negative). Relation between wave length and stimulation

WAVE LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS							RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY	
	Results of individual tests					Average			
	E. granulata		P. triqueter			E. gran.	P. triq.	E. gran.	P. triq.
432.6	0.6					0.6		0.759	
442.8	3.7	3.8		8.5	4.0	3.75	6.25	3.67	6.12
452.9	7.5	9.3	11.3	17.0	12.4	8.4	13.566	6.41	10.35
463.1	12.0	12.8	19.9	26.1	24.5	12.4	23.5	7.38	13.98
473.2	22.8	18.3	28.4	34.6	37.6	20.55	33.533	9.87	16.12
483.4	27.1	24.2	36.3	46.0	48.4	25.65	43.566	10.01	17.01
493.6	19.4	17.8	41.7	44.0	44.2	18.6	43.3	6.00	13.96
503.7	14.5	7.8	36.7	24.5	18.3	11.15	26.5	3.00	7.14
513.8		3.8		11.2	3.7(?)	3.8	7.45(?)	0.86	1.68
524.0				1.4(?)			1.4(?)		0.26(?)
Time	Aug. 25, 6.39–7.03 p.m.		Aug. 20, 3.42–4.01 p.m.						

Euglena granulata

Euglena granulata was discovered in one of the clay pools at Nela Park on August 25 but not in sufficient numbers to make work on it very satisfactory. Only one series of tests was made on the specimens collected at this time and the species was not again found during the season except here and there an isolated individual.

The results of the series of tests made may be found in table 6. This series of tests indicates that the distribution of stimulating efficiency in the spectrum for this species is the same as it s for the three species already discussed (see table 15).

Euglena minima

Euglena minima appeared at Nela Park in a pool containing much decaying grass. Here the organisms were collected in great abundance but they did not orient precisely enough to make it possible to obtain accurate results. A number of different tests was made and although the results obtained were very indefinite they seem to indicate that the stimulating efficiency of the different wave-lengths is the same as it is for the other species of Euglena tested (table 15).

The results of one series of these tests may be found in table 9. In this series the ocular slit was opened to 2.5 mm. so as to increase the illumination and the accuracy of orientation. This resulted in a very impure spectrum and consequently greatly reduced the value for comparative work of the readings.

Phacus triqueter

A considerable number of specimens of Phacus triqueter was found in liquid material collected in a pig-yard in Michigan near Ann Arbor (August 17). These specimens were fairly strongly negative, while most of the other organisms in the solution were either indifferent to light or positive. Thus it was possible in the course of a few days to isolate a sufficient number of them to make some fairly satisfactory readings in the spectrum. The results of these readings are given in table 6. This table shows that the relation between wave-length and stimulating efficiency in this form is practically the same as it is in Euglena (table 15).

Trachelomonas euchlora

On July 1 a small pool was discovered on an abandoned clay road in an open woods about half a mile south of the laboratory. The water in the pool was distinctly yellowish-green, due almost entirely to the presence of Trachelomonas euchlora. In the laboratory the specimens collected were found to be very strongly negative, but unfortunately they were not tested in the spectrum until July 29, when those in the laboratory, though still fairly numerous, responded much less precisely and those in the

TABLE 7

Trachelomonas euchlora (negative and positive). Relation between wave-length and stimulation

WAVE LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS									RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY	
	Results of individual tests							Average			
	Specimens positive Slits 0.75 mm. wide				Specimens negative. Slits 2.5 mm. wide			+	−	+	−
442.8						8.0			8.0		7.84
452.9	1.4		0.3			12.3		0.85	12.8	0.64	9.39
463.1	2.9	4.1	5.9	5.0	16.0	14.9		4.475	15.45	2.85	9.19
473.2	4.8	6.1	7.0	10.0	23.0	25.1		6.975	22.05	3.35	10.60
483.4	6.5	9.8	9.3	11.4	34.2	29.0	34.9	9.25	32.7	3.61	12.77
493.6	8.9	8.9	13.0	8.2	40.2	40.5	42.3	9.875	41.0	3.18	13.22
503.7	12.1	6.8	6.5	1.9	32.7	25.3	32.1	6.85	30.03	1.84	8.09
513.8	7.0	1.2			21.3		16.4	4.1	18.85	0.93	4.26
524.0	1.0				5.2		7.0	1.0	6.1	0.19	1.16
Time	Sept. 1, 4.40–6.00 p.m.				Sept. 1. 6.15–6.45 p.m.						

For description of table see legend table 2, p. 492.

pool had become scarce. The results obtained are consequently not very accurate. They indicate fairly clearly, however, that the distribution of stimulating efficiency in the spectrum is the same as it is for Euglena. Two of the best series of readings obtained are given in table 7. In one of these series the spectrometer slits were 2.5 mm., in the other 0.75 mm. wide. The table indicates that in the latter series the region of maximum stimulating efficiency was near wave-length 483 $\mu\mu$, that is, practically in the same position as it is for Euglena; but that in the former series it was farther toward the red end of the spectrum. This suggests the possibility of a Purkinje phenomenon. When, however, the two entire series of results are compared with similar series of results for Euglena it is found that there is a fair agreement in all, and that the shifting toward the red in the higher illumination is fairly definitely confined to the region of maximum stimulation, indicating that this is probably due to insufficient data.

Chlamydomonas (fluvialis and globulosa)?

Various species of Chalmydomonas were found in abundance at different times in the pools about the laboratory at Nela Park, but during the time the following tests were made they were not very abundant and orientation was rather indefinite, so that it was not possible to obtain very accurate results.

Two species were present in the culture studied. One was ellipsoidal in form with a maximum diameter of only about 6 μ, the other was nearly spherical and very much larger, having a diameter of approximately 30 μ. The former was probably C. fluvialis and the latter C. globulosa.

Most of the observations were made on the larger species, but both were continuously present in the test aquarium and the reactions in both appeared to be the same.

TABLE 8

Chlamydomonas sp? (positive). Relation between wave-length and stimulation

WAVE LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS							RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY	
	Results of individual tests					Average			
	A		B			A	B	A	B
473.2	16.0 (?)	19.0 (?)				17.5 (?)		8.41 (?)	
483.4	25.8	20.5	8.5		8.0	23.15	8.25	9.04	3.22
493.6	39.0	30.1	9.5	12.2	14.0	34.55	11.9	11.04	3.83
503.7	45.5	46.5	13.5	13.0	20.5	46.0	15.66	12.39	4.22
513.8	31 1	31.0		19.0	19.0	31.05	19.0	7.02	4.29
524.0	13.8	15.1		13.0		14.45	13.0	2.75	2.48
534.1				8.0			8.0		1.29
Time	Aug. 11, 1.28-1.45 p.m.		Aug. 2, 11.21-11.48 a.m						

For description of table see legend table 2, page 492.

From August 8 to 11 inclusive some specimens brought directly from the pools and others taken from solutions brought from the pools and kept in the laboratory were repeatedly tested in the spectrum under various conditions of illumination. They were found to be positive most of the time but orientation was usually so indefinite that only a few entire series of readings were obtained.

The results of two of the most extensive and accurate of these series are given in table 8. In the second series given in this table (B) the readings are much smaller than in the first (A). This is due to a great reduction in the luminous intensity of the spectrum caused by bending in the filaments of the lamp, which was not discovered until after the series of readings was completed. Both series indicate clearly that for the species tested the region of maximum stimulating effect and also efficiency is in the green between 500 and 515 $\mu\mu$, that is, considerably nearer the red end of the spectrum than it is for Euglena and Trachelomonas, but that the rate of decrease in stimulating efficiency in either direction from the maximum is about the same in all of these forms (table 15). This contention is supported by the results of all of the tests made on Chlamydomonas except those which were too indefinite to be of value.

The conclusions regarding the location of the region of maximum stimulating effect do not agree with that reached by Loeb and Wasteneys ('16). These authors maintain that this region is considerably nearer the red end of the spectrum, i.e., in the green near the yellow at about 535 $\mu\mu$.

The discrepancy between the results obtained by Loeb and Wasteneys and those obtained by the writer can not be accounted for on the basis of the fact that the former used a carbon arc as a source of light while the latter used a gas-filled tungsten lamp, for the difference in the distribution of energy in the two prismatic spectra is wrong in direction to explain this disagreement. The discrepancy mentioned must therefore b due either to the fact that the two sets of observations were not made on the same species, pisiformis being used by Loeb and Wasteneys and fluvialis (?) and globulosa by myself, or to inefficiency in methods and observations.

Gonium pectorale

Specimens of Gonium were frequently found in the clay pools at various times during the summer, but never in abundance and they were always mixed with other forms, so that the observations were continuously hampered by insufficient material, and moreover, orientation was never very precise. The results of

two of the most accurate and extensive series of readings obtained, one for negative and one for positive individuals, are presented in table 9. By referring to this table and table 15 it will be seen that these results show fairly conclusively that the distribution in the spectrum of stimulating efficiency for individuals when they are positive is the same as it is when they are negative; and that it is essentially the same for Gonium as it is for Euglena and Trachelomonas.

TABLE 9

Gonium pectorale (negative and positive). Euglena minima (negative)
Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS								RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY		
	Results of individual tests						Average				
	Gonium negative	Gonium positive		Euglena. Slit 2.5 mm. wide			G +	E −	Gonium −	Gonium +	Euglena −
442.8	8.9								8.72		
452.9	14.5		8.0	23.0			8.0	23.0	11.06	6.41	17.55
463.1	19.8		15.0	33.8	28.0		15.0	30.9	11.79	10.81	18.39
473.2	25.0		22.5	39.9	37.8	40.3	22.5	39.33	12.01	9.41	18.90
483.4	32.4	21.2	27.0	44.8	46.0	50.0	24.1	46.93	12.65	10.46	18.33
493.6	35.7	32.2	32.7	39.3	53.5	46.0	32.45	46.26	11.51	7.19	14.92
503.7	42.3	29.4	24.0	23.8	29.7	24.5	26.7	26.0	11.40	3.10	7.00
513.8	31.9	12.0	15.4	10.2			13.7	10.2	7.21	1.46	2.30
524.0	17.5	5.8	9.5				7.65		3.34	0.16	
534.1	2.7	1.0					1.0		0.43		
Time	Aug. 24 7.45–8.09 p.m.	Aug. 26, 4.36-4.58 p.m.		Aug. 27, 5.26–6.00 p.m.							

For description of table see legend table 2, p. 492.

Pandorina morum

Pandorina was found at Nela Park more nearly continuously, more widely spread and more abundantly throughout the sesaon than any other form studied. The colonies were often so numerous that the water in which they lived appeared distinctly green, and frequently the solutions in which they were found contained

scarcely any other organisms. There was consequently no lack of material for study. Moreover, these creatures respond very strongly to light and orient remarkably precisely, but there is one peculiar feature connected with their response that interfered considerably with the work.

This form is the first one that was studied and it was soon learned that while in a series of successive readings extending over a short period of time there was usually but little variation, there was often marked variation if the series was long continued, e.g., on July 27 in a series of readings the sector opening, with the wave-length of the colored light 524 $\mu\mu$, was 48.5 and 46.3 in two successive tests made at 8.37 and 8.39 a.m. respectively, while in two tests made about an hour later (9.35 and 9.36) under the same conditions of illumination the opening was 66.8 and 70.3 respectively. Similar incongruities were observed repeatedly in long continued series of tests.

Now, these variations were no doubt due in part to slight movement of the image of the luminous filament on the spectrometer slit resulting in variation in the illumination in the spectral beam as previously pointed out, but they were, I believe, more largely caused by the following peculiarity in the response of these organisms.

In a horizontal beam of light consisting of parallel rays, the colonies of Pandorina, like those of Volvox (Mast '07), do not swim directly toward or from the source of light but deflect to the left, and in a field of light consisting of two beams crossing at right angles, they also tend to deflect to the left so that in order to make them proceed on a line which bisects the angle between the beams, it is necessary to make the light in the beam to the right stronger than that in the beam to the left. This fact would not interfere in the least with the process of ascertaining the relative stimulating efficiency of the spectral colors by the method employed in this work if the tendency to deflect were continuously the same, but unfortunately it is not. The degree of deflection varies with the condition of the organisms and with the intensity of the light. The more strongly positive or negative the organisms are the less the deflection and conse-

quently the more nearly equal the stimulating effect of the light in the two beams is when the organisms follow a line bisecting the angle between the beams. The degree of positiveness or negativeness in these creatures varies with the intensity of the illumination and with the time they are exposed. In the beginning of a series of tests they usually orient more precisely and deflect less than they do later. We should consequently, under any given condition, expect the sector readings to increase, and this is precisely what was observed as stated above.

This difficulty was in part eliminated by taking for each individual test fresh specimens from a culture kept under constant conditions, and the most accurate readings were obtained in this way. The results of one such series of tests are given in columns 2–5, table 10, but such a series is by no means always obtainable, for very often the response of the colonies is too indefinite. This is clearly illustrated by the results of many preliminary tests made preceding those just mentioned as shown by the following abstract from my note book.

The specimens used in this series of tests selected to illustrate the point in question were collected late in the evening of August 11. They were very abundant and apparently in excellent condition. Two half-liter jars full of solution containing them were taken to the laboratory and placed in a north window. The following day colonies taken from these jars were repeatedly examined and tested under various conditions. Some were put into tap water, others into pond water; some were left in darkness, others in strong light, for various periods of time. Moreover, they were tested in illumination varying in intensity from very high to very low and in temperature varying from the maximum well toward the minimum, but in all cases orientation was too indefinite and movement too slow to obtain even moderately accurate results. Practically the whole time from early in the morning until three o'clock in the afternoon was spent in these fruitless attempts. During this time the two half-liter jars containing the colonies were continuously in the north window, but they were subjected to greatly varying conditions of illumination for this window was in the room in which the

TABLE 10

Pandorina (positive and negative). Relation between wave-length and stimulation

WAVE-LENGTH IN μμ	RELATIVE STIMULATING EFFECT IN TERMS OF SECTOR READINGS FOR PRISMATIC SPECTRUM												RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY		
	Results of individual tests									Average					
	Positive colonies				Negative colonies (A)			Negative colonies (B)		+	−A	−B	Positive	Negative (A)	Negative (B)
442.8	0.5	0.4	(?)	(?)				2.8	3.5	0.45		3.15	0.44		
452.9	1.6	1.5	1.5	1.7				4.6	2.3	1.56		3.45	1.19		2.63
463.1	2.6	3.2	2.8	2.4				5.5	5.9	2.75		5.70	1.63		3.39
473.2	4.4	6.3	4.3	6.8				8.0	8.0	5.45		8.00	2.62		3.84
483.4	6.2	7.0	8.9	9.2				10.2	10.5	7.825		10.35	3.05		4.04
493.6	10.1	12.5	15.3	13.9	10.5	11.5	11.2	11.4	14.2	12.95	11.066	12.80	4.17	3.57	4.13
503.7	19.1	16.6	19.5	19.8	18.0	19.5	19.9	29.0	26.8	18.75	19.133	27.90	5.05	5.16	8.32
513.8	27.3	27.8	26.3	29.0	20.5	34.7	23.4	33.7	34.0	27.6	26.2	33.85	6.26	5.92	7.67
524.0	34.0	36.9	33.5	36.2	19.3	37.0	31.4	48.5	46.3	35.15	29.233	47.40	6.70	5.57	9.04
534.1	36.8	39.5	35.7	41.0	31.2	42.8	37.5	61.7	63.8	38.25	37.166	62.75	6.17	5.99	10.12
544.3	42.0	42.0	43.4	44.3	27.8	48.7	47.0	71.4	57.6	42.925	41.166	64.50	5.89	5.65	8.85
554.4	37.0	40.2	39.6	43.8	37.3	39.1	38.4	59.0	64.6	40.15	38.266	61.80	4.75	4.52	7.31
564.5	27.9	30.3	32.1	32.5	26.6	30.0	27.8	46.6	57.2	30.7	28.133	51.90	3.14	2.88	5.31
574.6	24.5	20.9	26.2	27.3		16.8	24.0	52.4	59.5	24.725	20.4	55.95	2.19	1.81	4.97
584.8	13.7	15.5	15.0	13.7				40.0	36.0	14.475		38.00	1.13		2.96
594.9	6.2	6.5	9.4	7.5				21.8	19.6	7.4		20.70	0.51		1.42
605.0	3.4	3.7	3.1	3.3				11.0	11.4	3.37		11.20	0.20		0.68
615.2	1.0	1.7	(?)	(?)				6.9	5.0	1.35		5.95	0.073		0.32
625.3	0.9	0.5						4.3	3.3	0.7		3.80	0.03		0.18
635.4	(?)							2.5	1.5	(?)		2.00			.008
Time	August 12, 4.20–5.25 p.m.		August 12, 5.56–6.54 p.m.		August 9, 2.17–3.37 p.m.			July 27, 7.38–8.01 p.m.							

For description of table see legend table 2, p. 492.

tests were made; the opaque shades were frequently raised and lowered and the artificial light in the room was also repeatedly turned on and off. From three until four o'clock, however, the shades were continuously up and during this time the light was also stronger than it had been earlier in the day. After being thus exposed for an hour colonies taken from the jars were again tested and now, after having given very indefinite responses all day, they were, to my great surprise, very strongly positive and oriented remarkably precisely, and they continued to respond thus during a long series of tests, the results of which are among the most accurate and consistent obtained. They are given in columns 2–5, table 10.

In these tests fresh colonies were taken after every second reading. One pipette-full of tap water and one-half pipette full of water from the jars containing numerous colonies were put into the observation aquarium, then a reading was made in a given color and the result recorded in column 2. The solution in the observation aquarium was now thoroughly stirred and another reading made in the same color and the result recorded in column 3, after which the wave-length was changed by 10 $\mu\mu$, fresh colonies put into the aquarium and the process repeated. All of the readings in this series of tests were made in this way, and thus variation in the degree of deflection was largely eliminated. This is shown by the remarkably close agreement in the results obtained at different times in the same wave-lengths and recorded in table 10.

In this table are also recorded the results obtained in two of the best series of observations made with colonies in the negative state. By examining this table, table 15, and figure 4, in which some of the results are plotted, it becomes evident at once that the distribution of stimulating efficiency in the spectrum is strikingly different for Pandorina and Euglena, Chlamydomonas or any of the other forms previously discussed. The region of maximum efficiency is considerably nearer the red and the effective part of the spectrum extends from the maximum in either direction much farther. The curve representing the results obtained with Pandorina is consequently considerably

flatter than those representing the results obtained with Euglena or with Chalmydomonas. In Euglena the effective region extends approximately from 420 to 520 $\mu\mu$, while in Pandorina it extends approximately from 440 to 620 $\mu\mu$. And the region of maximum stimulation for Pandorina, which is in the green in the neighborhood of 524 $\mu\mu$, nearly coincides with the upper limit of the effective region for Euglena; so that the stimulating efficiency for Euglena is practically zero in the region which has the highest stimulating efficiency for Pandorina.

By comparing the last three columns in table 10 with the three preceding columns it will be seen that while the region of maximum efficiency is, as previously stated, near 524 $\mu\mu$ the region of maximum effect is near 544 $\mu\mu$. That is, the correction for unequal distribution of energy and dispersion in the spectrum resulted in a considerable shift of the maximum toward the blue, a much greater shift than similar corrections produced in Euglena. This is, of course, due to the fact that in the former the region of maximum stimulating effect is nearer the red than in the latter. Further reference to these tables and to figure 4 will also indicate that, just as in Euglena, a change in the sense of reaction does not alter the relative stimulating efficiency of the spectral colors; it appears to be the same regardless as to whether they are positive or negative.

The conclusion reached on the basis of the results of the three series of tests given in table 10 are in the main supported by the results obtained in practically all of the other series of tests made, a total of 24. Many of these series, however, were limited to the region of the spectrum extending but a short distance on either side of the maximum and the results obtained in them consequently bear primarily on the question as to the location of this point.

By referring to table 10 it will be seen that the point of maximum stimulating effect in all of the three series of tests recorded lies near wave-length 544 $\mu\mu$. In 11 of the 24 series not recorded in this table it was found to be in practically the same place as in those recorded in the table, in 9 somewhat nearer 554 $\mu\mu$, in one very near 564 $\mu\mu$ and in the remaining 3 somewhere

between 524 and 574 $\mu\mu$, the results being too indefinite to locate it more precisely. In three of these series the colonies were negative and in two of these the region of maximum effect was near 554 $\mu\mu$, while in the remaining one it was near 544 $\mu\mu$. These results support the contention that the relation between stimulating efficiency and wave-length is the same for negative as it is for positive colonies.

In three of the series of tests the spectrometer slits were reduced to 0.1 mm., thus greatly increasing the purity of the spectrum and greatly reducing the illumination. In all of these series the effective region in the spectrum was greatly shortened, this region extending only approximately from 510 to 570 $\mu\mu$. In two of these series the region of maximum effect was near 544 $\mu\mu$, but in the other one it was nearer 554 $\mu\mu$. There is consequently no evidence indicating that increase in the purity of the spectrum causes any appreciable alteration in the location of this region or that it is shifted toward the violet in low intensity as it is in the case of human beings. That is, there is no evidence indicating that there is a Purkinje effect in Pandorina.

The distribution of maximum efficiency was calculated only for the results obtained in the three series of tests given in the table, but there is no reason for assuming that such a calculation for the remaining series of tests would yield results materially different.

Eudorina elegans

Eudorina and Pandorina were usually found in the same pools, but both were never observed to be very abundant at the same time. Pandorina ordinarily predominated when the pools contained much decaying organic material, and Eudorina when they contained little. Thus in fresh pools rich in such material the former generally appeared first, developed rapidly until a certain stage in the disintegration of the organic substance was reached and then they gradually decreased, while the latter appeared and began to increase, becoming abundant after the former had practically disappeared.

Eudorina orients very precisely in light, but like Pandorina it deflects to the left and the degree of deflection varies with the con-

ditions of the colonies, making it difficult to obtain accurate results in long continued series of tests. The colonies are sometimes negative and sometimes positive, but during the time that they were studied most extensively (July 31 to August 12) they were never found to be very strongly negative, so that satisfactory series of results were obtained only with positive colonies.

The results of two of the most successful of the series obtained are presented in table 11, columns 5, 6, and 7. By comparing these results with those obtained in Pandorina it will be seen that the distribution in the spectrum of stimulating effect and stimulating efficiency are essentially the same for the two forms mentioned (see table 15). The regions of maximum effect and maximum efficiency appear to be in the same locations in both, and the extent of the effective regions in either direction from these locations appears to be about the same.

A comparison of the results of all of the tests made, however, indicates that the maximum for Eudorina is slightly nearer the violet than it is for Pandorina. In the 11 series made with Eudorina the region of maximum stimulating effect was found to be between wave-lengths 534 and 554 $\mu\mu$ in all. In 5 of these series it was very near 534 $\mu\mu$, in 4 near 544 $\mu\mu$, and in 2 the precise location was questionable, while in the 27 series of tests made with Pandorina the region of maximum effect was found to be, as previously stated, near 544 $\mu\mu$ in 14, near 554 $\mu\mu$ in 9, near 564 $\mu\mu$ in 1, and not precisely located in 2. Thus the maximum was near 534 $\mu\mu$ in nearly half of the tests made with Eudorina and in none made with Pandorina, while it was near 554$\mu\mu$ in one-third of the tests made with Pandorina and in none made with Eudorina.

Spondylomorum quaternarium

The colonies of Spondylomorum used in these experiments were collected on August 27 in Michigan near Ann Arbor in a yard frequented by pigs. They were immediately taken to Cleveland, and during the following three days they were tested from time to time in various conditions of illumination. In some of the tests traces of acid were added and the temperature was

TABLE 11

Eudorina elegans (positive); spondylomorum quaternarium (positive and negative). Relation between wave-length and stimulation

WAVE-LENGTH IN μμ	RELATIVE STIMULATING EFFECT IN TERMS OF SECTOR READINGS FOR PRISMATIC SPECTRUM							RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY			
	Results of individual tests					Average					
	Spondylomorum			Eudorina		Spon.	Eud.	Spon.	Spon.	Eud.	Eud.
	Negative	Positive		A	B	+	A	−	+	A	B
422.4					1.4						2.33
432.6					1.6						2.02
442.8		2.3		0.6	2.8	2.3	0.6		2.25	0.58	2.74
452.9		4.0		1.4		4.0	1.4		3.05	1.06	
463.1		7.3		2.4	8.9	7.3	2.4		4.34	1.42	5.29
473.2		11.3	11.8	4.5	10.0	11.55	4.5		5.55	2.16	4.80
483.4		18.0	15.5	10.0	23.7	16.75	10.0		6.54	3.90	9.25
493.6		22.5	18.7	18.2	28.1	20.6	18.2		6.64	5.87	9.06
503.7	21.8	28.8	28.5	24.5	43.0	28.65	24.5	5.87	7.72	6.60	11.59
513.8	32.5	34.5	34.9	39.6	44.0	43.7	34.7	41.8	7.37	7.85	9.45	9.88
524.0	41.4	40.8	40.0	45.5	57.3	59.3	40.4	51.4	7.90	7.71	9.80	11.31
534.1	61.5	51.3	48.9	69.0	69.2	85.1	50.05	69.1	9.91	8.07	11.14	13.72
544.3	68.5	58.0	48.3	53.0	65.6	73.7	53.15	59.3	9.40	7.28	8.14	10.12
554.4	47.5	48.2	39.5	45.2	49.5	74.0	43.85	47.35	5.62	5.18	5.60	8.75
564.5	38.5	26.3	28.4	34.5	35.8	53.8	27.35	35.15	3.92	2.80	3.60	5.51
574.6	31.5	22.2	23.0		31.0	23.4	22.6	31.0	2.80	2.00	2.75	2.08
584.8	20.9	14.0	16.4		17.8	15.0	15.2	17.8	1.63	1.18	1.39	1.17
594.9	8.9	11.7	15.2		12.3	12.5	13.45	12.3	0.61	0.92	0.84	0.86
605.0		9.0	13.3		5.5	5.6	11.15	5.5		0.68	0.33	0.34
615.2		8.9	11.3		3.8	5.5	10.1	3.8		0.54	0.20	0.29
625.3		6.5	10.0		1.9	2.3	8.25	1.9		0.40	0.09	0.11
635.4		4.0			1.0		4.0	1.0		0.17	0.04	
645.6		1.5			0.6	0.5	1.5	0.6			0.02	0.02
Time.	August 30, 1.31–1.44 p.m.	August 30, 1.48–2.25 p.m.		August 7, 10.04–11.01 a.m.		August 7, 11.11–11.56 a.m.					

For description of table see legend table 2, p. 492.

raised and lowered. Sometimes they were found to be negative and sometimes positive, but during all of this time orientation was rather indefinite excepting for short periods until in the afternoon of the third day, when the colonies were found to be very strongly positive and orientation was fairly precise. The only extensive series of tests obtained with Spondylomorum was made at this time. The results of this series are given in table 11, columns 2, 3 and 4.

A comparison of these results with those obtained with Pandorina and with Eudorina (table 15) shows that the relation between stimulation and wave-length is essentially the same for all. Moreover the results obtained in short series of tests made with negative colonies indicate that the response is the same as it is in positive colonies, just as it was found to be in Pandorina and a number of other forms.

Arenicola cristata

The larvae of Arenicola used in the following observations were obtained from eggs, expressed from Woods Hole, Mass., to Cleveland. The eggs were shipped September 16 and received September 18. The following day there was a considerable number of larvae apparently in excellent condition. They were strongly positive and oriented accurately but they deflected somewhat to the right. This accounts for the low results obtained as compared with those obtained in observations on Euglena under the same conditions.

Several series of tests were made during the day. In the first four the spectrometer slits were 0.75 mm. wide and in the last two 2.5 mm. wide. All of the results obtained in these tests are given in table 12. The next day the larvae were again tested but they were found to be too sluggish and their responses too indefinite for successful work.

By referring to table 12 it will be seen that the four series of results obtained in the first set of tests are nearly alike, and that the regions of maximum stimulating effect and maximum stimulating efficiency lie respectively in the blue between wave-lengths 483 and 493 $\mu\mu$, very near wave-length 483 $\mu\mu$. It will

TABLE 12

Arenicola larvae. Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS								RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY	
	Results of individual tests						Average			
	Spectroscope slits 0.75 mm.				Slits 2.5 mm.		Slit 0.75 mm.	Slit 2.5 mm.	Slit 0.75 mm.	Slit 2.5 mm.
432.6						1.6		1.6		2.02
442.8	1.4	1.0	?		3.3	1.9	1.2	2.6	1.17	2.54
452.9	3.7	2.8	3.2	3.5	5.3	6.0	3.3	5.65	2.52	4.31
463.1	6.5	5.4	3.5	4.4	17.3	10.2	4.95	13.75	2.94	8.18
473.2	9.0	6.4	6.3	7.8	23.0	20.8	7.375	21.90	3.54	10.52
483.4	11.7	10.2	10.4	11.9	28.1	19.6	11.05	23.85	4.31	9.31
493.6	11.3	8.8	11.1	14.6	22.2	19.3	11.45	20.75	3.69	6.69
503.7	9.5	6.4	7.0	13.0	20.1	13.8	8.975	16.95	2.42	4.57
513.8	7.0	4.9	6.0	6.9	12.5	10.4	6.2	11.45	1.40	2.59
524.0	3.0	3.0	3.3	2.8	9.0	6.0	3.025	7.5	0.57	1.43
534.1	1.6	?	?	0.5	6.8	6.0	1.05	6.40	0.17	1.03
544.3						3.7		3.70		0.51
554.4						2.3		2.30		0.27
564.5						0.8		0.80		0.08
Time	Sept. 19, 1.00–1.49 p.m.			Sept. 19, 3.01–3.59 p.m.	Sept. 19, 4.23–5.33 p.m.					

For description of table see legend table 2, p. 492.

also be seen that the distribution in the spectrum of stimulating efficiency is throughout very much like that in Euglena, the maximum being located almost precisely in the same place for both (table 15). There is, however, some evidence indicating that the effective region extends somewhat farther toward the red in the former than it does in the latter. It will be seen, moreover, that in the tests made with the spectrometer slits wider and the illumination consequently stronger the maximum is somewhat nearer the violet. This is just the opposite from what would be expected in case there were a Purkinje effect.

Loeb and Wasteneys ('16, p. 232) maintain that in a "carbon arc spectrum" the maximum stimulating effect for Arenicola larvae is "situated in the bluish-green in the region of about

$\lambda = 495\ \mu\mu$," that is, considerably nearer the red than I found it to be in a tungsten prismatic spectrum. This difference can not be accounted for on the basis of difference in the distribution of energy in the spectra used for the carbon arc spectrum both prismatic and normal (Loeb and Wasteneys do not state which they used) contains relatively more energy in the region of the shorter waves than the tungsten prismatic spectrum. We should therefore expect the region of maximum effect to be nearer the red in the latter than in the former in place of the opposite. The difference noted must consequently be due to inaccuracies in observation or defects in methods.

Lumbricus terrestris

In the experiments on the earthworm and the blowfly larvae precisely the same methods were used as in those on the other forms studied. The observation aquarium was, however, replaced by a ground glass plate 21 x 21 cm., on which the animals were exposed. This plate was covered with a sheet of wet filter paper in the experiments on the earthworms and with a coat of olive oil in those on the blowfly larvae. In both cases the surface of the glass plate thus treated proved to be an excellent substratum for the animals to travel on, and this is very essential in work of this sort.

To test these animals it was necessary to have a larger field of light than was used in the observations on the microscopic forms. The distance between the center of the field and the ocular slit of the spectrometer was consequently increased to 53 cm. This caused a marked reduction in the intensity of the colored beam of light, which made it necessary to also reduce the intensity of the white beam. This was done by moving the source to a distance of 100 cm. and by interposing a ground glass plate, thus making the illumination in the white beam at the center of the field with the sector open 7.4 (?) m. c. The two beams of light, both of which were horizontal, crossed at right angles at the center of the glass plate and at this point each of them was 6 cm. wide.

The glass plate contained a heavy black line which passed through the center and bisected the angle between the two beams of light; and, just as in the preceding experiments, the illumina-

tion in the white beam of light was adjusted until the animal in each case followed this line. To accomplish this it was often necessary to make them travel across the field several times for each color tested. This was done by letting them crawl onto a piece of tin of the proper size at the end of the course and then gently transferring them to the beginning and letting them crawl off again.

The specimens[3] used in the observations on Lumbricus were very small, averaging only about 4 cm. in length. All of them were found in a small area on a sidewalk after a heavy rainstorm (September 26). After reaching the laboratory they were kept continuously in darkness until used in the observations, all of which were made the following day. The results obtained in these observations are presented in table 13. The averages of these results corrected for unequal distribution of energy in the spectrum are presented in the last column of this table.

TABLE 13

Lumbricus terrestris. Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS									RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY
	Result of individual tests								Average	
463.1	1.5		1.1		1.0	1.5	2.5	1.3	1.483	0.882
473.2	3.3	3.2	2.3		2.0	3.3	3.0		2.85	1.370
483.4	9.2	6.8	6.0		8.9	6.4	6.3		7.266	2.838
493.6	9.2	6.8	8.5		5.0	10.0	8.3		7.966	2.569
503.7		4.0	5.0	2.5	2.5	5.2	5.4		4.1	1.105
513.8		1.0	2.0	1.4		1.9	1.0		1.46	0.330
Time	Sept. 27, 11.25 a.m. to 2.10 p.m.		Sept. 27, 2.45-6.51 p.m.			Sept. 28, 4.26-6.35 p.m.				
Size of worm	4 cm. long		3.5 cm. long			4.5 cm. long				

For description of table see legend table 2, p. 492.

[3] Some of these specimens preserved in formalin were examined by Prof. Frank Smith and identified as very young Lumbricus terrestris. I am greatly indebted to Professor Smith for his efficient service.

These results indicate that the distribution in the spectrum of stimulating efficiency for Lumbricus is essentially the same as it is in the Arenicola larvae, Euglena, Phacus, Trachelomonas and Gonium (table 15). The location of the region of maximum efficiency corresponds fairly well in all and the fact that the total effective region is somewhat shorter for Lumbricus than for the other forms is no doubt due to the difference in the illumination used in the experiments, it being considerably lower in those on Lumbricus than in those on the other forms.

Allolobophora foetida was also tested but only a few times and the responses were always rather indefinite, but the results obtained indicate that the relation between stimulation and wavelength in this form is the same as it is in Lumbricus terrestris.

Blowfly larvae

The blowfly larvae used in the following work were obtained by exposing pieces of raw beef. No attempt was made to identify them. They were kept continuously in very weak light or in darkness and all individuals which did not orient fairly accurately were discarded.

A total of 12 series of tests was made (September 18 to 23) with larvae in various stages of development. In the first four the collimator slit was 1 mm. wide and the ocular slit 2.5 mm. wide. In the next seven both slits were 1 mm. wide and in the last both were 0.5 mm. wide. There was no marked difference in the results obtained under these various illuminations. Taken as a whole they were not very satisfactory and much more accurate results could undoubtedly be obtained with the method employed if more time were devoted to the work.

The results show clearly, however, that the distribution of stimulating efficiency is more nearly in harmony with that found in Chlamydomonas than with that found in any of the other forms studied. The results obtained in three of the most satisfactory series of tests made are given in table 14 and the averages of these three series, corrected for unequal distribution of energy, are plotted in figure 4. These results indicate that the regions of maximum stimulating effect and efficiency are respectively

TABLE 14

Blowfly (larvae). Relation between wave-length and stimulation

WAVE-LENGTH IN $\mu\mu$	RELATIVE STIMULATING EFFECT OF DIFFERENT REGIONS IN PRISMATIC SPECTRUM IN TERMS OF SECTOR READINGS				RELATIVE STIMULATING EFFICIENCY CALCULATED ON THE BASIS OF EQUAL ENERGY
	Results of individual tests			Average	
422.4	0.1			0.1	0.16
432.6	1.4			1.4	1.77
442.8	0.8(?)			0.8(?)	?
452.9	4.7	8.3		6.5	5.00
463.1	8.2	13.0	19.5(?)	10.6	6.31
473.2	22.3	20.0	25.0	22.433	10.78
483.4	27.5	33.0	36.4	32.3	12.61
493.6	39.8	40.5	40.0	40.1	12.93
503.7	57.3	43.2	54.5	51.666	13.92
513.8	56.3	59.6	56.5	57.466	13.00
524.0	54.9	57.9	57.2	56.666	10.81
534.1	56.3	56.2	52.9	55.133	8.89
544.3	46.3	42.0	50.2	46.166	6.31
554.4	25.7	37.9	33.0	32.2	3.81
564.5	22.7	16.1	28.0	22.26	2.28
574.6	14.4		11.5	12.95	1.15
584.8	9.0			9.0	0.70
594.9	5.0			5.0	0.34
605.0	4.5			4.5	0.27
615.2					
625.3	2.8(?)			2.8(?)	0.13
Time	Sept. 22, 10.16–11.05 a.m.	Sept. 23, 10.01–10.58 a.m.			
	Slit 1 mm.	Slit 0.5 mm.			

For description of table see legend table 2, p. 492.

located between wave-lengths 513 and 524 $\mu\mu$, very near 503 $\mu\mu$, apparently somewhat nearer the red than these regions are in Chlamydomonas. The total effective region also appears to extend somewhat farther toward the red in the former than in the latter. In the whole 12 series of tests made the region of maximum effect was found to be between wave-lengths 510 and 540 $\mu\mu$ in all but one. In seven of these the location of this region was very near 530 $\mu\mu$, in three near 520 $\mu\mu$, and in two it was questionable.

TABLE 15

Relative stimulating efficiency of different regions in the spectrum for fifteen species; reduced to the same magnitude and averaged so that they can be readily compared. Maximum in heavy type

	WAVE-LENGTH IN $\mu\mu$	EUGLENA VIRIDIS		EUGLENA GRACILIS		EUGLENA TRIPTERIS	EUGLENA GRANULATA	EUGLENA MINIMA	PHACUS TRIQUETER	TRACHELOMONAS EUCHLORA		GONIUM		ARENICOLA LARVAE	LUMBRICUS	CHLAMYDOMONAS	BLOW-FLY	PANDORINA		EUDORINA	SPONDYLOMORUM	
		Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Positive	Negative
	422.4	2.50				1.64											.24			(?)		
Blue	432.6	4.09	4.29		2.76	7.12	1.52										2.65			(?)		
Blue	442.8	10.41	7.69	6.43	7.70	12.78	7.34		7.22	11.91		13.95		5.49			(?)	1.32		(?)	5.62	
Blue	452.9	13.24	12.70	12.27	16.06	15.54	12.82	18.60	12.21	14.27	3.55	17.69	11.90	11.84			7.50	3.57	5.26	(?)	7.62	
Blue	463.1	14.86	16.66	16.43	17.45	17.38	14.76	19.49	16.49	13.96	15.81	18.86	16.60	14.01	6.26		9.46	4.89	6.98	4.63	10.85	
Blue	473.2	18.22	18.30	20.09	19.60	18.13	19.74	**20.03**	19.02	16.11	18.59	19.21	**20.10**	16.63	9.72		16.17	7.86	7.68	5.49	13.87	
Blue	483.4	**20.40**	**20.86**	**20.16**	**20.10**	**20.00**	**20.02**	19.42	**20.07**	19.41	**20.03**	**20.24**	17.50	**20.24**	**20.14**	14.83	18.91	9.15	8.04	10.26	16.35	
Green	493.6	14.46	18.30	12.01	13.47	13.37	12.00	15.81	16.47	**20.09**	17.64	18.41	19.45	17.34	18.23	17.96	19.39	12.51	10.09	11.89	16.60	
Green	503.7	8.23	9.46	4.38	4.69	4.81	6.00	7.42	8.42	12.29	10.21	18.24	13.37	11.37	7.84	**19.88**	**20.88**	15.15	16.93	14.40	19.30	11.85
Green	513.8	3.06	3.13	0.54 (?)	1.04	1.06	1.72	2.43	1.98	6.47	5.16	11.53	5.76	6.58	2.34	15.70	19.50	18.78	17.55	15.71	19.62	14.88
Green	524.0	1.08	1.00		0.51	0.15			0.30 (?)	1.76	1.05	5.28	2.71	2.67		8.01	16.21	**20.10**	18.34	17.07	19.27	14.95
Green	534.1											0.68	0.29	7.99			13.33	18.51	**20.12**	**20.04**	**20.17**	**20.01**
Green	544.3																8.41	17.67	18.28	14.71	18.20	18.98
Green	554.4																5.71	14.25	14.85	11.42	12.95	11.35
Yellow	564.5																3.42	9.42	10.11	7.26	7.00	7.91
Yellow	574.6																1.72	6.57	7.99	3.99	5.00	5.65
Yellow	584.8																1.05	3.39	5.92	2.10	2.95	3.29
Red	594.9																0.51	1.53	2.84	1.38	2.30	1.23
Red	605.0																0.40	0.60	1.36	0.97	1.70	
Red	615.2																	0.22	0.64	0.39	1.35	
Red	625.3																0.19	0.09	0.36	0.16	1.00	
Red	635.4																		0.16	0.07	0.42	
Red	645.6																			0.03		

The results taken as a whole consequently support the contention that the relation between wave-length and stimulation in the blowfly larvae is more nearly like that in Chlamydomonas than like that in any of the other organisms studied but that the longer waves are somewhat more effective in the former than in the latter (table 15).

Gross ('13) made a very thorough study of the blowfly larva with reference to the relative stimulating efficiency of four different spectral colors, blue (420–480 $\mu\mu$), green (490–550 $\mu\mu$), yellow (570–620 $\mu\mu$) and red (630–650 $\mu\mu$). In this study he forced the larvae to enter the side of a field of light consisting of two horizontal beams with rays opposite in direction and different in color but equal in energy. Under these conditions the beam from which they turn is, of course, the more efficient of the two in which they are exposed. By thus testing successively various combinations, Gross found the order of efficiency in the four colors tested to be green, blue, yellow, red. These results are in full harmony with those presented above, as can be clearly seen by referring to table 15 and figure 4.

DISCUSSION

The results obtained show clearly that the reactions in all of the species studied are dependent upon wave-length, certain colors are much more efficient as stimulating agents than others, but they are not wholly dependent upon wave-length, for while there is clearly a region of maximum stimulating efficiency in the spectrum, stimulation is not confined to this region and the stimulating effect of the wave-lengths on either side of it can be made greater by simply increasing their intensity. There is consequently no evidence in the results obtained indicating the presence of color-vision in any of the forms studied, for it is the absence of any such relation between reactions and intensity that constitutes the chief objective characteristic of color-vision.

Moreover, the fact that there is no variation in the distribution of stimulating efficiency in the spectrum dependent upon different physiological states, it being the same in specimens, e. g.,

either in the negative or in the positive state, is not what would be expected in organisms with color-vision. The relation between color and stimulation is of the same order in all of the fifteen species studied as it is in color-blind human beings, and in one of the former (blowfly larvae) it is in fairly close agreement with that of the latter.

The lowest forms in which color-vision has been clearly established are, in my opinion, honey bees. Both Lubbock ('95) and Frisch ('14) have shown fairly conclusively that these insects can be trained, in gathering honey, to select any one of a considerable number of different colors regardless of their luminous intensity. The conclusion of Frisch and Kupelwieser ('13, p. 552) that Daphnia has a sense of color (Farbensinn) is in my opinion not well founded. These authors demonstrated that under certain conditions Daphnia is positive in red, yellow and green, and negative in blue-green, blue and violet, i.e., that change from any of the former to any of the latter has the same effect as an increase in luminous intensity; and that a change in the opposite direction has the same effect as a decrease in luminous intensity. This seems to indicate merely that these two groups of colors are antagonistic in their effect, just as has been found to be true for similar groups in their effect on certain photochemical reactions and on the reactions of some other organisms (Mast, '11, pp. 310, 335).

Loeb and Wasteneys ('16) conclude that the relation between wave-length and reactions is the same in animals as it is in plants and that there are two different "types of photosensitive substances" in both. Neither of these conclusions is well founded. The first conclusion is based largely upon the fact that the relation mentioned above is not the same for all plants, just as it is not the same for all animals. But it would be quite as logical to conclude the opposite, for in some plants the shorter wave-lengths are relatively more efficient as stimulating agents than they are in any animals (see table 15), and in none of the plants is the entire visible spectrum effective, nor are there any in which the reactions depend upon the wave-length to such an extent as they do in those animals which have color-vision.

The second conclusion is based upon the contention that the region in the spectrum of maximum stimulating efficiency is either in the blue or in the green for all organisms. This is probably not true, but even if it were true it would be just as logical to conclude, on the basis of the results obtained by these authors, that there are three or four types of photosensitive substances, for they maintain that the maximum is respectively at 460–490, 495, 535, 560–578 $\mu\mu$ for Euglena, Arenicola, Chlamydomonas and Balanus. Our results and those of others also indicate that the maximum is located in several different regions and that some of these do not correspond with any of those given; consequently if the argument of Loeb and Wasteneys is valid there are at least four "types of photochemical substances." This argument appears to be valid, however, only if the relative absorption of the different colors is the same in those organisms in which the location of the maximum differs.

All that can be said, then, regarding the chemical reactions associated with reactions to colors is that they differ in all organisms for which the distribution of stimulating efficiency in the spectrum differs, provided there is no difference in selective absorbtion. It can not be said, however, that the chemical reactions are the same in all organisms in which the spectral distribution of stimulating efficiency is the same, for, judging from our present knowledge of photochemistry, which is admittedly very inadequate, the relation between photochemical reaction and wave-length is in many instances the same for different substances. The fact that the relation between wave-length and reaction is the same for individuals when they are negative as it is when they are positive supports this contention, for the chemical reactions associated with these different responses are, in all probability, not the same.

SUMMARY

1. The distribution in the spectrum of the stimulating effect and the stimulating efficiency in reference to energy was ascertained for fifteen species, including unicellular and colonial forms, worms and fly larvae.

2. In all of the species studied stimulation by light was found to depend upon the wave-length, i.e., certain spectral colors are much more efficient as stimulating agents than others. In the spectrum from the region of the maximum the stimulating efficiency decreases rather rapidly in either direction and the effective region is much shorter than the visible spectrum.

3. There is no evidence indicating that stimulation in any of the species studied is independent of luminous intensity, for if the light in the spectrum on either side of the maximum be made sufficiently intense it becomes more effective than that at the maximum. This holds also for the reaction of plants to light and probably for all photochemical reactions. There is consequently no evidence indicating the presence of color-vision in any of the forms studied. Bees are the lowest form in which color-vision has been clearly established.

4. The distribution in the spectrum of stimulating efficiency differs in some species that are closely related (Gonium and Pandorina) and is essentially the same in some that are not closely related (Euglena and Lumbricus). The region of maximum efficiency is near 483 $\mu\mu$ for Euglena, Trachelomonas, Phacus, Gonium, Arenicola, and Lumbricus; near 524 $\mu\mu$ for Pandorina, Eudorina and Spondylomorum; near 503 $\mu\mu$ for Chlamydomonas and blowfly larvae; near 465 $\mu\mu$ for green plants and somewhat nearer the red for fungi.

5. It consequently differs for plants as well as for animals, but the shorter wave-lengths are relatively more efficient for green plants than they are for any animals and there is nothing in the nature of color-vision in any of the plants. The contention therefore that the reactions to colors in plants and animals is the same is not well founded, although some of the chromatic reactions in animals may be essentially the same as those in plants.

6. The distribution in the spectrum of stimulating efficiency in any given species is continuously the same, regardless of changes in physiological states, environment and character of response, e.g., it is the same in individuals when they are negative as it is when they are positive.

7. As to the nature of the chemical processes associated with the responses no definite conclusions can be drawn; but if the absorption is the same in the photosensitive tissues of all of the organisms studied these processes differ in all species in which the relation between wave-length and stimulation differs. It can not, however, be concluded, even if this is true, that they are the same in all of the species in which this relation is the same, for the relation between chemical reactions and wave-length is, in all probability, the same for certain substances that differ. Further progress in this analysis depends largely upon further work on the relation between photochemical reactions and wave-length.

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THE EFFECTS OF A THYROID DIET UPON PARAMAECIUM

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TWELVE FIGURES

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INTRODUCTION

The classical investigations of Maupas ('88–'89) upon the life cycle of Paramaecium, with the subsequent researches of Calkins and of Woodruff, have established the value of the division-rate as an index to the vitality of pedigreed races. Calkins ('15) concludes, "that more or less definite cycles of vigor or depression, ending in natural death unless conjugation or its equivalent supervenes, are characteristic of all pedigreed races of infusoria." It has been shown by many investigators that this life cycle, as indicated by the division-rate is readily influenced by environmental changes, i.e., in the temperature, amount or kind of food present, or chemical composition of the culture medium.

Certain substances which produce decreases in the rate of division have been carefully studied by Woodruff (summary and literature list, 1912). While on the other hand several substances have been reported to produce the opposite effect, less evidence has been adduced either as to the effect or its nature.

In 1912 I commenced feeding experiments upon Paramaecium, using the substance of the thyroid gland. In 1914 I was able to show that this substance produced great increases in the division rate of a pedigreed race of Paramaecium aurelia. In that paper I reviewed experiments of Nowikoff ('08), who had arrived at somewhat similar conclusions through a different method of investigation. Since that time Budington and Harvey ('15) have reported experiments using the thyroid substance from fish, amphibia, reptiles, birds and mammals which confirm the results I obtained from mammalian tissue.

Meantime I have carried my investigations further with a view to confirming my results on Paramaecium aurelia by similar experiments on P. caudatum; to ascertain whether the effect produced by the thyroid is unique among the internally secreting glands and by what fraction of the thyroid it is produced, what effect continued thyroid feeding might have upon the life cycle, and what other effects on the activities or structures of Paramaecium might be discovered bearing on the nature of the thyroid effect. While definite answers to all these queries have not yet been obtained, the data collected may be brought together at this time, and certain conclusions obtained.

The experiments here reported have been carried on in the Zoölogical Laboratory of Columbia University, the Marine Biological Laboratory at Woods Hole, and the Biological Laboratory of Amherst College. For the preparation of desiccated glands I am indebted to the Research Laboratory of Organotherapeutics of Armour and Company. From my colleagues at Amherst I have received many helpful suggestions in the preparation of this report. I take this opportunity, finally, to express my sincere thanks to Prof. Gary N. Calkins, at whose suggestion these experiments were commenced and whose critical advice has assisted their prosecution.

METHODS

The experiments here reported have been performed on three races of Paramaecia whose life histories are given in full below. These races were in each case derived from a single individual and thereafter maintained in four files by daily isolations following the method described by Calkins ('02). Each individual was placed in a solid shallow watch glass in four drops of the culture medium and at the end of twenty-four hours (or rarely a longer period) the number of Paramaecia present was obtained and the number of divisions calculated. An individual selected at random was then isolated in the same manner, using a pipette reserved for the line in question. Records were also kept of the number of deaths and monstrosities occurring. At the end of every five days the total number of divisions in the four files of each line was obtained and averaged to give the average division rate per day of the line for that period. These five day averages form the units for our discussion.

The culture media employed for the control lines were in all cases hay infusions. The formulae for these will be cited under the life histories of the three races. The thyroid and other glandular media were prepared in different experiments as emulsions of fresh glands from rabbits, rats, and cattle, and as suspensions of commercial gland preparations. The following preparations of Armour and Company were employed: desiccated thyroids U. S. P. (sheep), thymus desiccated (calf), pituitary body desiccated (ox), desiccated suprarenal capsules (?), pancreas desiccated (pig), spleen desiccated (calf), ovarian substance desiccated (pig). Experiments were also conducted with iodothyrin (combined with sugar of milk), a commercial preparation of Friedr. Bayer and Company. Formulae used are cited under each experiment.

Experimental lines were in all cases derived by isolating one individual from each file of the control line, thus obtaining two lines of maximum similarity. Where several lines were required as in some experiments, the four files of the control were allowed to multiply until the required number was present in each, and

then isolated to establish the several lines required. All the customary precautions against contamination were observed, and every attempt made to keep the control and experimental lines under conditions exactly similar except for the kind of culture medium employed. All the watch glasses were stacked in a single large moist chamber and the number of Paramaecia in each was ascertained at the same time.

The cultures were carefully examined for monstrosities and the later history of many of these followed. In cases where a single individual required a closer study than could be obtained with a binocular or the lower power of the microscope, the following procedure was adopted. A ring of vaseline was turned on a slide, the individual in question was placed in a minute drop on a coverslip, the drop drained off with a fine capillary tube until there was barely enough left for the individual to move, the coverslip was then inverted upon the ringed slide and the individual could be studied under high power for a considerable time and finally returned to the watchglass. After this technique was perfected, no ill-effects were produced on the Paramaecia so treated. Permanent preparations were made from time to time by fixing in Bouin's fluid (picro-aceto-formol) and staining with Delafield's haematoxylin according to the following formula, for which I am indebted to my friend Professor Haughwout:

Delafield's haematoxylin conc. aq. sol	9 cc.
Glacial acetic acid	1 cc.
Distilled water	90 cc.
Chloral hydrate	½ gr.

HISTORY OF THE RACES

The life history of the three races of Paramaecia which have been studied are summarized in the following paragraphs.

Race A. Paramaecium aurelia. Descended from an individual isolated March 12, 1913, from a mass culture which had been maintained in the Zoölogical Laboratory at Columbia University for several months. This race was kept in pedigreed culture until December 20, 1913, when it died in a typical depres-

sion period after a life history of 420 generations. The control medium used was one-half strength standard hay infusion, prepared by boiling 1 gram of hay for ten minutes in 100 cc. of tap water and allowing it to stand exposed to the air for twenty-four hours, when it was mixed with an equal quantity of boiled tap water. The life history of this race as shown in the daily division rate averaged by ten day periods is given in figure 1.

Race B. Paramaecium caudatum. Descended from an individual isolated July 2, 1914, from a stock culture of the Marine Biological Laboratory. This race was maintained as a pedigreed culture until October 18, when a pair of conjugants was isolated from an epidemic of conjugation which had been induced in stock at about the 150th generation. From an individual resulting from the third division after the ex-conjugants had separated, or in other words, when reorganization was completed, a new line B′ was established.

Line B′. This line was maintained in a pedigreed culture until January 6, 1915, when it died out in a depression period, 81 generations after conjugation and 230 (approximately) after the original isolation. An experiment with boiled thyroid was in progress at this time, and from this line after thirty days of thyroid treatment a new line B″ was established.

Line B″. This line was maintained similarly until May 31, 1915, when it died out in transportation from New York. It had been maintained for 159 generations after thyroid feeding was discontinued and 460 after the original isolation of the race. The control medium employed for all these lines was prepared by boiling approximately 0.5 gram of hay seed in 50 cc. of spring water, filtering and using it after cooling. Figure 2 shows the life history of this race by ten day periods.

Race C. Paramaecium caudatum. On July 27, 1915, a conjugating pair was isolated from an epidemic of conjugation induced in stock from the Zoölogical Laboratory of Smith College. Two divisions after separation one of the quadrants was isolated. From this individual a pedigreed culture was established and has been maintained up to the date of writing when it stands in the 172nd generation. The control medium used for this race

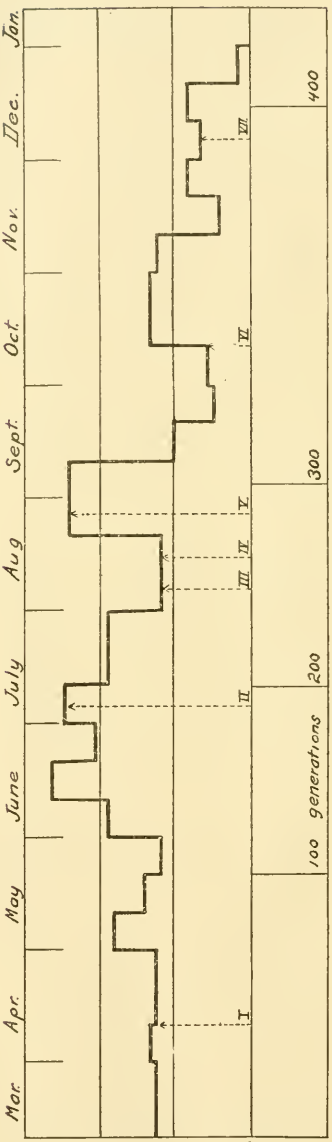

Fig. 1 Graph showing life history of the A race in average daily division rate reaveraged by ten day periods. Roman numerals designate the points at which the experiments cited in the text were commenced.

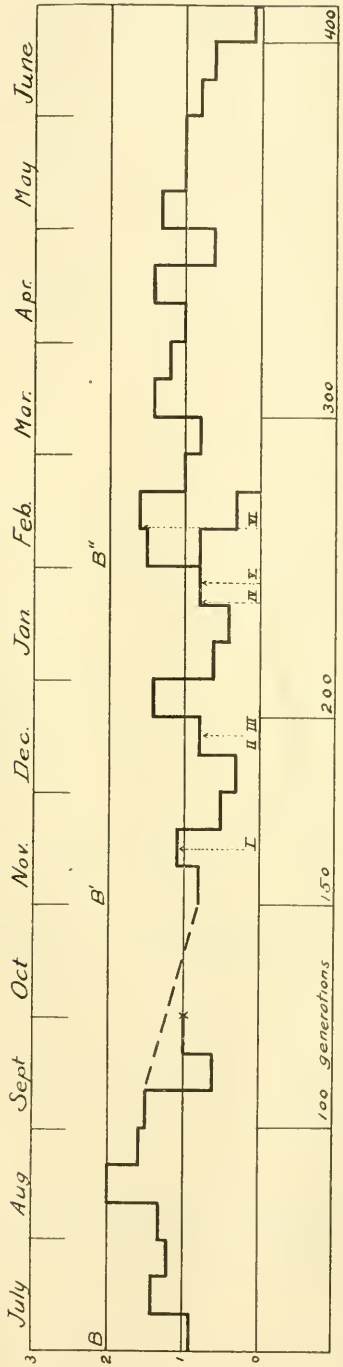

Fig. 2 Graph showing the life history of the B race in average daily division rate re-averaged by ten day periods. Roman numerals designate the points at which the experiments cited in the text were commenced.

has been one-half strength standard hay infusion used after cooling. The life history as averaged for ten day periods is shown in figure 3.

In this connection it may be pointed out that the last two races, B′ and C, were derived from a single reorganized individual of an endogamous conjugant and present protoplasm as homogeneous as possible. It will also be observed that these conjugations serve as logical starting points for the life histories, a point of considerable importance for experiments such as these, in view of the fact that the protoplasm of Paramaecium is in different conditions at different stages of the life history.

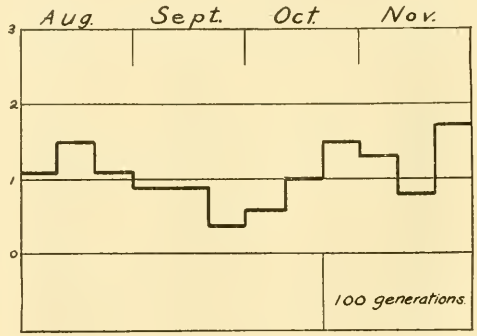

Fig. 3 Graph showing life history of the C race in average daily division rate re-averaged by ten day periods.

FEEDING EXPERIMENTS

a. On the A race. Nine experiments on this race of Paramaecium aurelia in which parallel lines were maintained on hay infusion and thyroid media are here cited (tables 1 to 9); a summary (table 10) is added for comparison with the results obtained with Paramaecium caudatum. The relation of the different experiments to the life cycle of the race is shown in figure 1, where the points at which the experiments were begun are indicated by roman numerals.

Experiment A 1 was initiated by extracting two lines from the control and treating one daily with thyroid, the other with thymus. These gland preparations were from three sources, *a*,

TABLE 1

Race A. Experiment 1. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS	THYROID-CONTROL
7	1.50	2.70	0.55	
8	1.05	1.95	1.10	
9	1.00	2.90	1.10	
10	1.55	3.60		1.90
11	1.45	2.80		1.40
12	2.20	3.15		2.25
13	0.95	2.15		
14	1.75	2.60		
15	1.00	2.40		
Average.....	1.40	2.80	0.90	1.85

from the freshly dissected glands of ten rabbits macerated and mixed one part to two parts boiled tap water. The emulsions were kept in a refrigerator at about 10°C. and used during period 7, in the proportion 2 drops to 2 drops of the control hay infusion. A second source, *b*, was from twenty rats similarly prepared and used during period 8, as above and during periods 9–12 in the proportion $\frac{1}{4}$ drop to 4 drops of hay infusion. The third source, *c*, was from nine rabbits prepared and used as above during periods 13–15. The thyroid and thymus emulsions were prepared similarly from the same animals and were kept and used under identical conditions. After it was evident that the thyroid line was dividing more rapidly than the control (an effect not produced by the thymus) a line was extracted from the thyroid treated one and returned to control medium in which it returned to the average division rate shown by the control line.

TABLE 2

Race A. Experiment 2. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS
10	2.15	3.15	1.00
11	1.20	2.80	
12	2.50	3.10	
Average.....	1.65	3.10	

Experiment A 2 was commenced while the previous experiment was yet in progress, to discover whether other individuals of the A race were susceptible to the thyroid effect. An individual was selected from the A line and allowed to divide until there were twelve cells of the same generation. Four of these were continued as a control line, four treated with thyroid and four with thymus.

TABLE 3

Race A. Experiment 3. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS
23	2.65	3.20	2.15

Experiment A 3, performed after a railroad journey to Woods Hole and a change to the local tap water, was continued for only one period owing to the difficulty of keeping fresh tissue, in this case from cattle glands supplied me through the kindness of Dr. Gudernatsch.

TABLE 4

Race A. Experiment 4. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID
23	1.70	2.90
24	2.10	3.50
Average.....	1.90	3.20

Experiment A 4, performed at the same time as A 3 and under the same conditions, had as control a line which had been treated with thyroid for nine periods and thereafter maintained in the control medium for eight periods.

TABLE 5

Race A. Experiment 5. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYROID-CONTROL	THYMUS
31	0.70	3.70	1.75	
32	1.70		1.80	1.80
33	2.25		2.50	2.00

Experiment A 5 was carried on during a depression period at a time when the control line was reduced to two individuals.

One of these was maintained in the control medium, where it continued four days without division and then slowly returned to the control rate. Another was treated with a suspension of Armour's thyroids (prepared by shaking up 1.5 mgm. in 2 cc. boiled tap water and mixing with 2 cc. control hay infusion) where it divided twice in the ensuing twenty-four hours. Two of the resulting cells were returned to the control medium and table 5 shows the comparative rates of division of the control, thyroid and thyroid-control during the next few periods. A thymus line started during this experiment using Armour's thymus (prepared like the thyroid suspension) shows the same rate of division as the control.

TABLE 6

Race A. Experiment 6. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID
32	1.70	1.85
33	2.25	3.70
Average.....	2.00	2.55

Experiment A 6 was carried on as the race began to emerge from the depression period. Thyroid prepared as in the previous experiment was used and after one period of slight effect produced a very large increase in the division rate.

TABLE 7

Race A. Experiment 7. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS
34	2.50	3.10	2.50
35	2.30	3.25	2.00
36	2.40	3.25	2.95
Average.....	2.40	3.20	2.50

Experiment A 7 was performed during a time when the race was at its highest division rate, and is rendered especially significant by the fact that eight files were maintained for each line instead of four. It will be noticed in this and other experiments where the desiccated thymus was employed that the lines so

treated did not show the decrease in division rate that is observable in the earlier experiments, a decrease due to the increase in death rate produced at the same time.

TABLE 8

Race A. Experiment 8. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS
46	1.50	2.65	1.30

Experiment A 8 carried on after return to New York from Woods Hole and after an interval during which observations were made at irregular intervals (one to four days) resulted in the regular increase in division rate after thyroid feeding.

TABLE 9

Race A. Experiment 9. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS
54	0.65	1.00	0.80
55	0.70	1.40	1.15
56	0.95	0.70	1.05
57	0.40	0.20	0.15
Average.....	0.70	0.85	0.75

Experiment A 9 was performed during the final depression period which carried off the race. The individual cells at this time gave evidence of their condition not only in lowered division rate, but in decreased size, more sluggish movements and the appearance of monstrosities. Thyroid feeding produced no effects after the first two periods and the thyroid treated line succumbed more rapidly than the control. All attempts to save the race by other food media, chemicals and increasing the amount of culture media failed.

These experiments showed that the thyroid fed individuals divide more rapidly than those kept in hay infusion no matter whether the race was at a high or low point in the life cycle. The only exception observed was during the final depression period which carried off the race. They also show that thyroid indi-

TABLE 10

Race A. Summary of experiments. Average division rate per day

EXPERIMENT	CONTROL	THYROID	DAYS
I	1.40	2.80	35
II	1.65	3.10	15
III	2.65	3.20	5
IV	1.90	3.20	10
V	0.70	3.70	5
VI	2.00	2.55	10
VII	2.40	3.20	15
VIII	1.50	2.65	5
IX	0.70	0.85	20
Average.....	1.60	2.65	120

Percentage increase produced by thyroid feeding, 65 per cent.

viduals were less liable to death from slight environmental changes. At all times the thyroid fed individuals were more active, smaller and more transparent. Paramaecia treated with thymus showed no such effects. In the course of these experiments, some thyroid particles were stained with an alcoholic solution of Congo red, the free color washed out in distilled water. These prepared thyroid particles were then fed to Paramaecia in the manner customary in these experiments. It was possible to observe the formation of gastric vacuoles containing the prepared thyroid and later to note the change from an acid to an alkaline reaction shown by the indicator, exactly as described by Métalnikow ('12).

b. On the Brace. In the summer of 1914 attempts were made to discover whether the effects produced by thyroid feeding were due to the iodine content of the gland. It was found that iodine and iodine in combination with potassium iodide resulted in depressing rather than raising the division rate even when used in such small doses as 1/20,000,000. The experimental data are given in table 11.

In the fall of this year experiments were undertaken to discover whether the effect of the thyroid is unique among the internally secreting glands in producing an increased rate of division. In the first experiment cited, four lines were derived from the control B′ and treated for five periods of five days

TABLE 11

Race B. Experiments with iodine. Average daily division rates re-averaged by five day periods

PERIOD	CONTROL	IODINE n-2000
5	1.35	0.95
6	1.30	1.10
7	1.40	1.30
Average.....	1.35	1.10

Experiments with iodine and potassium iodide. Protocol

Primary solution iodine, 1, potassium iodide, 2, water 300

Solution iodine 1–2000	Death immediately
1–20,000	Death in two or three minutes
1–200,000	Death in 48 hours
1–2,000,000	Death in 72 hours
1–20,000,000	Decrease in division rate

Average daily division rates re-averaged by five day periods

PERIOD	CONTROL	IODINE	IODINE-CONTROL
26	2.15	1.15	
27	1.90	1.05	
28	1.60	1.30	1.75
Average.....	1.90	1.15	

each in a medium of 2 drops of hay infusion plus 2 drops of the desired gland suspension (prepared by shaking up 2 mgm. of the desiccated gland substance in 2 cc. of boiled tap water). The glands used were thyroid, suprarenal, thymus, and pituitary.

TABLE 12

Race B. Experiment 1. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	ADRENAL	THYMUS	PITUITARY
4	1.15	1.85	1.95	1.55	2.00
5	0.70	2.00	1.75	1.05	1.60
6	0.30	0.65	0.75	0.70	0.75
7	0.25	1.75	1.40	1.70	1.70
8	0.50	2.00	2.10	2.10	2.20
Average.....	0.60	1.65	1.60	1.40	1.65

The experiment was unfortunately interrupted during period 6, when I was called out of town. During this period the lines were kept in hay infusion and not isolated daily. Table 12 contains the daily division rate averages by five day periods.

It will be seen from these figures that all the lines divided more rapidly than the control, with practically no differences between them. This experiment is the only one giving such a result and by consulting the life history of the B race, figure 2, it will be seen that the race is passing through the low point of a rhythm.

TABLE 13

Race B'. Experiment 2. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	THYMUS	PANCREAS	SPLEEN	OVARY	SUPRA-RENAL	PITUITARY
10	1.55	1.65	9.50	0.35	0.30	0.70		
11	1.30	1.45	0.50	0.25	0.30	0.60		
12	0.80	0.90					0.20	0.55
13	0.30	0.85						0.00
Average	1.00	1.20	0.50	0.25	0.30	0.65	0.20	0.55

In order to secure a more crucial test it was determined to keep the experimental lines in the gland suspension alone, i.e., without the addition of hay infusion. Experiments were performed on seven lines, using the following glands: thyroid, thymus, pancreas, spleen, ovary, suprarenal and pituitary bodies, with the result shown in table 13.

Under these conditions the thyroid line alone survived after a few days treatment, presenting on the contrary active individuals at all times and a division rate consistently a little higher than the control. In order to discover whether this effect was permanent the thyroid line was continued from this time on till January 26, 1915, when it died out in the 101st generation since conjugation, on the same day on which the control line died out. The five day averages of the daily division rate are shown in table 14. Figure 4 shows graphically the life history of the control and thyroid lines during this period. The close similarity of the two curves is worthy of mention. It will be ob-

TABLE 14

Race B′. Experiment 3. Division rate averaged by five day periods

PERIOD	CONTROL	THYROID
10	1.55	1.65
11	1.30	1.45
12	0.80	0.90
13	0.30	0.85
14	0.30	0.85
15	0.45	1.30
16	0.65	0.95
17	1.00	1.20
18	1.05	1.60
19	0.50	1.10
20	0.25	0.20
21		
Average.....	0.75	1.00

served that though the thyroid line shows a consistently higher division rate than the control it was unable to survive the depression period.

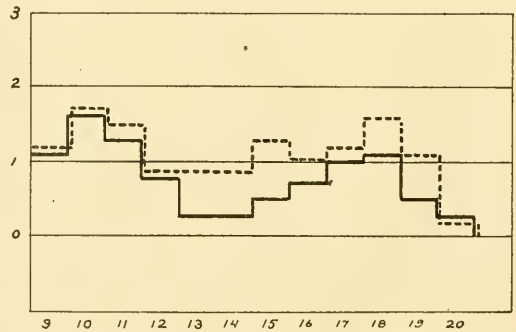

Fig. 4 Graph showing comparative life histories of the thyroid fed and control lines during Experiment B3 in average daily division rate re-averaged by five day periods. Control ——, thyroid - - - -.

Experiments were now begun in the hope of localizing the factor to which the thyroid effect was due. Solutions of the commercial iodothyrin were prepared in the same manner as the glandular media. Table 15 shows the division rates obtained from the control, thyroid, and two iodothyrin lines. It will be

TABLE 15

Race B'. Experiments with iodothyrin. Average daily division rate averaged again by five day periods

PERIOD	CONTROL	THYROID	IODOTHYRIN I	IODOTHYRIN II
25	1.45	2.00	1.55	1.45
26	1.25	2.00	1.35	1.00
Average.....	1.35	2.00	1.45	1.25

seen that the iodothyrin produced no effect at a time when the thyroid treatment brought about an increase of 0.65 divisions per day.

Other experiments were conducted with weaker solutions of the iodothyrin which gave essentially the same results. It having been suggested that boiling the thyroid would test the question as to whether the effects produced were due to an hormone, experiments along this line were begun. A 2 grain tablet of thyroid substance was boiled for an hour in 100 cc. of spring water and filtered. The residue was evaporated by slow drying. Approximately 2 mgm. of the dried substance was mixed in 2 cc. of spring water to make a culture medium in which a line of Paramaecia was maintained for five days by the usual method. Another line was similarly treated with the filtrate (thyroid bouillon). The results are shown in table 16.

TABLE 16

Race B'. Experiment 4. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	BOILED THYROID	THYROID BOUILLON
17	1.00	1.20	1.20	1.00

TABLE 17

Race B'. Experiment 5. Daily division rate averaged by five day periods

PERIOD	CONTROL	THYROID	BOILED THYROID
18	1.05	1.60	1.10
19	0.50	1.10	1.90
20	0.25	0.20	1.75
Average.....	0.60	1.00	1.25

To test this point still further another experiment was performed in which a line was maintained on boiled thyroid prepared by boiling the usual thyroid suspension for ten minutes and adding boiled water to supply that lost by evaporation. The control line and the thyroid line of Experiment B 3 were continued during this experiment until the life cycle of the B′ line came to an end, the thyroid line after sixty days of treatment dying out at the same time. The division rates by five day periods are shown in table 17.

TABLE 18

Race B″. Experiment 6. Daily division rate averaged by five day periods

PERIOD	BOILED THYROID	THYROID-CONTROL
21	1.95	1.35
22	1.40	0.70
23	1.35	0.80
24	1.35	0.80
25	2.00	1.45
26	2.00	1.25
27	1.60	1.25
28	2.05	1.15
29	1.80	0.95
30	1.70	0.95
31	1.95	1.15
32	1.75	1.65
33	1.30	0.40
34	1.70	0.80
35	1.70	1.50
36	2.20	1.10
37	2.00	1.10
38	1.50	0.95
39	2.30	0.80
40	1.75	1.10
41	1.85	1.10
42	1.70	0.50
43	0.90	0.40
44	1.15	0.70
45	0.85	0.10
46		
Average.....	1.65	0.95

The line which had been drawn from the control only fifteen days before and treated with boiled thyroid continued at a high rate of division while the control and thyroid lines cited above were undergoing their last depression period. From this another line B″ was drawn and returned to the control medium. Table 18 gives the division rate of these two lines for the following one hundred and twenty-five days, during which the line treated with boiled thyroid divided at a rate of 0.70 of a division per day more rapidly than the line derived from it and subsequently maintained in hay infusion. And in figure 5 are shown graphically the life histories of these two lines, where again the close similarity of the two curves may be observed.

TABLE 19

Race B. Summary of experiments. Average division rate per day

EXPERIMENT	CONTROL	THYROID	DAYS
I	0.60	1.60	25
II	1.00	1.20	25
III	0.75	1.00	60
IV	1.00	1.20	5
V	0.60	1.25	20
VI	0.95	1.65	125
Average.....	0.85	1.40	260

Percentage increase produced by thyroid feeding, 64 per cent.

Finally in table 19 is given a summary of the average daily division rate of the control and thyroid lines respectively obtained in all the experiments performed with race B. A comparison with table 10 shows that the effect of the thyroid is not at first sight so marked in the caudatum race (an increase of 0.55 division per day) as in the aurelia race (an increase of 1.05 divisions per day). This difference disappears when we compare the average division rates of the control lines for the two species (0.85 and 1.60 divisions per day respectively) and we find that the *percentage of increase in the two races is practically identical, being 65 per cent for Race A and 64 per cent for Race B.*

c. On the C race. The third race of Paramaecia, C, was established in order to observe in greater detail the other effects

produced in thyroid fed individuals. Records were also kept for the purpose of comparison with the effects produced on the division rate. A summary of these reduced to five day averages is given in table 20 for the control, a thyroid line. The results are similar to those obtained in the other two races and it will be noted that the percentage increase produced by the thyroid is again 65 per cent. A graph (fig. 6) comparing the life histories of the thyroid and control line illustrates how closely the control life curve follows that of the control, *points of high and low rates of division occurring simultaneously notwithstanding the fact that at these points the thyroid line is many generations older.*

TABLE 20

Race C. Average daily division rate re-averaged by five day periods

PERIOD	CONTROL	THYROID
2	0.80	0.60
3	1.60	1.10
4	1.40	1.40
5	1.30	1.60
6	0.80	1.40
7	1.10	2.00
8	0.70	2.30
9	0.60	2.90
10	1.10	1.60
11	0.40	1.00
12	0.30	0.90
13	0.40	1.10
14	0.70	1.40
15	0.70	1.90
16	1.30	2.90
17	1.80	2.70
18	1.20	2.10
19	1.60	2.30
20	1.00	1.60
21	0.70	1.80
22	0.95	1.95
23	1.70	1.85
24	1.85	1.80
25	1.25	1.25
Average for 125 days ..	1.00	1.65

Percentage increase produced by thyroid feeding, 65 per cent.

THE EFFECT OF THE THYROID

a. On the division rate. In my experiments on Paramaecium aurelia (Race A), a small quantity of thyroid emulsion was added to the hay infusion which is the common laboratory culture medium for this form, and a sharply marked increase in division rate resulted. No such increase was observable in the similarly treated thymus lines. The same method was tried in the first experiments on the caudatum race (B 1) but did not give satisfactory results. Since that time I have conducted my experiments with media prepared by shaking up the gland desiccations in spring water. Many and long continued experiments have demonstrated that the thyroid tissue (together with the inevitable bacterial flora) under these conditions presents all the elements necessary for the maintenance of life in these infusoria. I emphasize this statement in view of my demonstration of the ingestion and digestion of thyroid particles by Paramaecia. Other internally secreting glands either do not supply these elements or form in decomposition (as some of them undoubtedly do) substances lethal to the individuals treated with them (Experiment B 2).

The thyroid however contains some substance which causes the Paramaecia to divide more rapidly. Experiments have demonstrated that in both species tested the thyroid feeding has produced an increase in the division rate of 65 per cent. In other words a Paramaecium dividing once in twenty-four hours in hay infusion would give rise to 1024 daughter cells in ten days; the same Paramaecium if treated daily with thyroid would produce about 185,000 daughter cells.

Attempts have been made to identify this substance in iodothyrin (table 15). These have failed, perhaps because the preparation (commercial iodothyrin in sugar of milk) was not sufficiently active. I have not yet had the opportunity of testing a pure iodothyrin. Other experiments have been conducted with iodine, pure or combined with potassium iodide (table 11). Far from causing any increase in division rate these preparations proved toxic, causing decreases in the division rate even in the minimal effective dosage.

Experiments with boiled thyroid (tables 16, 17, and 18) have demonstrated that it produces effects similar to those of the raw and desiccated gland substance. The agent at work is not extracted by boiling as shown by parallel experiments with the filtered bouillon. It may be noted in this connection that Oliver and Schäfer ('95) reported that boiled thyroid produced the same depressant effect on heart action as the untreated gland. If the results obtained in these experiments are due to a hormone secreted by the thyroid it must be one of remarkable stability.

Attention is called to the constancy with which the effect of the thyroid has been observable during extended periods of daily treatment (figs. 4, 5, and 6). In the longest individual experiment (one hundred and forty days) the thyroid fed line divided more rapidly than the control in every period. Nor has this rapid rate of division produced any harmful effects. In no case has a thyroid fed line died out before its control.

At many different periods lines have been instituted from thyroid lines of long standing and abnormally high division rates, returned to the control medium and continued as parallel lines to the parent thyroid and control lines (Experiments A 1, A 5, B 6). In every case the thyroid-control line slowly returned to the normal (hay infusion) rate of division. No unusual rise in the death-rate marked the transfer. It may be remarked in this connection that the transfer from hay infusion to thyroid media is also unaccompanied by any unusual mortality.

It is of particular interest to observe the effects of the thyroid at times in the life history when the race is undergoing a depression period. It has been shown by Calkins ('04) that a number of different factors may be involved in carrying a line through this critical period. He has demonstrated that the mechanical agitation of a railroad journey, a sudden change of diet, a difference in the salt content, or an increase in temperature may be sufficient to restore a weakened line to its normal vitality. According to his interpretation this power of inducing regeneration is the essential feature of conjugation. The recent suggestive observations of Woodruff and Erdmann ('14) show that the

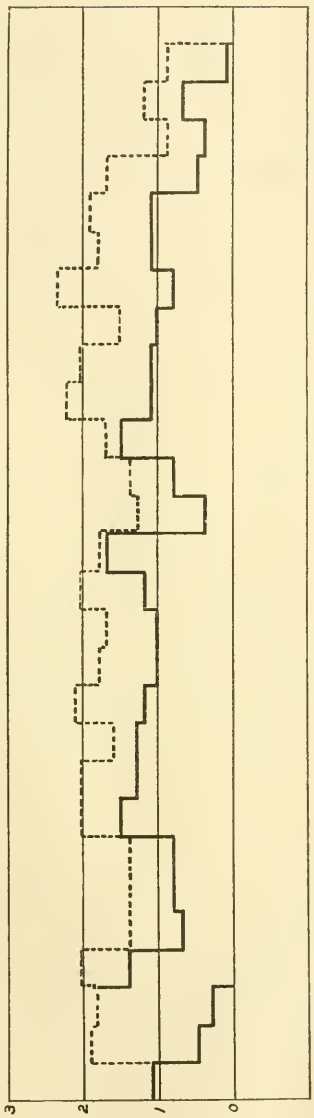

Fig. 5 Graph showing comparative life histories of the thyroid fed and control lines during Experiment B6 in average daily division rate re-averaged by five day periods. Control ——, thyroid - - - -.

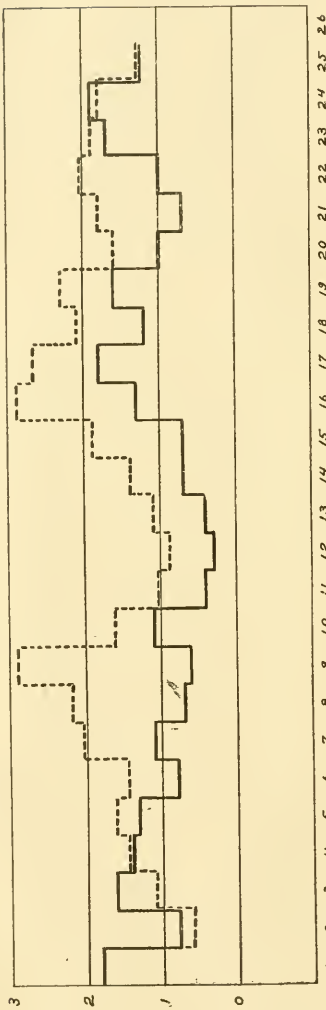

Fig. 6 Graph showing comparative life histories of the thyroid fed and control lines during Experiment C, in average daily division rate re-averaged by five day periods. Control ——, thyroid - - - -.

race may in some cases rejuvenate itself by a peculiar process of parthenogenesis (endomixis).

The cyclical character of the life histories of my three races is shown by figures 1, 2, and 3. A few cases where experiments with thyroid were in progress at depression periods may now be considered. Experiment A 5 was commenced shortly before the beginning of a depression period (fig. 1) where for four days there was but one individual in the control line. A line which had been derived from this a few days previously and treated with thyroid divided during this period at a rate of three and seven-tenths divisions per day. This and other experiments have indicated that initial stimulation with thyroid results in carrying the race at a high rate of division through a depression period from which the control line recovers with great difficulty if at all. So in Experiment B 5 (fig. 5) during a similar depression period the control line died out, while a line which had been recently treated with boiled thyroid went through the depression period with a division rate higher than normal.

In this same experiment however a thyroid line which had been under treatment for a considerable period died out at the same time as the control. Referring to Experiment A 9, we find that in the depression period that carried off the race, a thyroid line of twenty days treatment, which at first had produced an increase in division rate, succumbed at the same time as the control.

Comparing the life cycle curves of thyroid fedand control lines (figs. 5 and 6), we note that in these two experiments, each over three months in duration, the thyroid fed line, though always at a much higher division rate shows the same rhythms as the control. Much of this similarity is of course due to similar environmental causes, changes in temperature, etc. But it is very striking that although the thyrold line is many generations older than the control line, the periods of depression occur at the same times. These experiments further indicate that the maximum effects of the thyroid treatment are produced at points where the division rate of the control is at its maximum.

b. On the gastric vacuoles. The gastric vacuoles, those 'improvised stomachs' in which the processes of digestion take place, have been carefully studied by many investigators. Métalnikow ('12) has shown that in any given line of Paramaecia, the rate of formation of these vacuoles is constant under normal conditions, subject to an half-hourly rhythm and not influenced by the quantity of food present. The quality of the food however exercised a striking effect on the rate of vacuole formation. A more easily assimilable food produced a decrease in the time intervals between the formation of the vacuoles and an increase in the length of their cyclosis. Experiments with changed environmental conditions showed that this effect was also produced by slight doses of arsenic and alcohol and by raising the temperature. As these agents have been reported as increasing the division rate of Paramaecium, it seemed advisable to test the effect of thyroid feeding with this index.

The technique employed is a modification of that of Métalnikow. The Paramaecia left over in the watch glasses after the daily isolations were allowed to multiply for not over three days in small test tubes containing 4 cc. of hay infusion or thyroid suspension. A large number of Paramaecia was removed from each test tube and allowed to stand half an hour in ten drops of freshly prepared hay infusion or thyroid suspension. Twenty or more individuals from each culture were isolated on two slides and a drop of carmine solution added. After five minutes they were killed and fixed by adding on the underside of a coverslip the following fluid.

Glycerin	10 cc.
Corrosive sublimate	to sat.
Glacial acetic acid	1 cc.
Camphor water	10 cc.

This fluid kills rapidly with a minimum of distortion and clears the protoplasm so that the carmine filled vacuoles are remarkably distinct and easy to count. The number of these vacuoles is counted for twenty individuals on each slide and an average obtained. These slides may then be laid aside until opportunity permits of verifying the count.

After a few preliminary experiments on the B race had given negative results, the C race was established especially for these experiments. On the whole the results obtained were negative. A few individuals from the thyroid line formed an abnormally large number of vacuoles in the five minute test: and equally large number formed an abnormally small number of vacuoles. On the whole the averages obtained from the counts of many individuals were alike. In table 21 are given summaries of a few of the experiments performed on Race C. Here are recorded the period in which the experiments were performed, the number of carmine filled gastric vacuoles formed in the first five minutes of feeding by the control and thyroid lines, and, for comparison, the percentage increase in division rate produced by the thyroid during the same periods.

TABLE 21

Race C. Number of gastric vacuoles formed in the first five minutes of carmine ingestion

PERIOD	CONTROL	THYROID	PERCENTAGE INCREASE IN DIVISION RATE
15	3.0	5.0	171
16	4.0	5.0	123
18	2.0	2.5	75
19	1.5	1.0	43

This table will serve to show that while there may be an increase in the rapidity of gastric vacuole formation in the thyroid fed lines, it is not in the least comparable with the increase in the rapidity of division produced. In this connection it will be recalled that whereas Woodruff and Baitsell showed that increases in the temperature produced within certain limits increases in the rapidity of the division rate directly conformable to van't Hoff's law, Métalnikow studying the effect of the temperature on the rate of formation of the gastric vacuoles was unable to bring his results under this same law, the increases in rapidity of vacuole formation being in all cases too small.

c. On the contractile vacuoles. One of the most striking features of these experiments was the discovery, in the thyroid fed line of the C race, of Paramaecia with an abnormally large num-

ber of contractile vacuoles. The first observation was made by one of my students on an individual from a mass culture descended from my thyroid fed line. Since that time, September 25, 1915, I have been searching my thyroid and control lines for individuals with peculiarities of the excretory system. I find Paramaecia with three contractile vacuoles in my thyroid fed lines with great regularity, usually between 20 and 30 per cent of all the individuals observed being characterized by the extra vacuole. In spite of the most painstaking observations I have never found them either in the control line nor in the mass culture from which the C race was established, and I am satisfied that there exists a definite causal relation between the appearance of these abnormal Paramaecia and their thyroid diet.

The small number of forms actually observed may be due to a form of division in which two vacuoles go to one of the daughter cells while the other will have but one until the normal *de novo* formation of the second vacuole. If then the extra vacuole is not formed for a short time, both Paramaeca will appear to have two contractile vacuoles, even though there be a predisposition in one (or both) to form a third. Thus our observed number of trivacuoled forms will be smaller than the expectation.

Shortly after my attention had been turned to these remarkable individuals, Hance's brief notice in *Science* ('15) appeared describing similar phenomena in Paramaecia which had been previously immunized to very high temperatures. Hance reports the occurrence of Paramaecia with as many as six contractile vacuoles but concludes "The only definite statement that may be made of this race is that it has a tendency to more than two contractile vacuoles." He suggests that this potentiality for extra vacuoles may have been acquired under the stress of the unusual environment (a temperature of 40°C.).

I have not found Paramaecia with more than three vacuoles. The third lies midway between the other two organoids and pulsates in rhythm with the others. In all other characteristics these trivacuoled forms appear perfectly normal. Figures 7 and 8 represent two of these Paramaecia from my thyroid line after more than a hundred generations of treatment.

While an increase in the number of contractile vacuoles was the most striking effect produced by the thyroid on the excretory system, other derangements of greater or less importance have been observed. In the forms with three vacuoles the apparatus of each has appeared normal, as has been the rate of pulsation. In thyroid treated individuals with but two vacuoles, however, there have always been abnormalities of some sort present. In some cases these appear as an increased rate of pulsation, in others as an increase in the number of feeding canals to from eight to ten, whereas the normal number is five or

Fig. 7 Paramaecium caudatum after 180 days thyroid treatment, showing three contractile vacuoles. Drawn from life.

Fig. 8 Paramaecium caudatum after 180 days thyroid treatment, showing three contractile vacuoles. Drawn from life.

six. In still others the feeding canals, instead of forming a star around the vacuole, are greatly elongated to form systems stretching the extent of the cell.

These abnormalities occurring in the excretory system of the thyroid fed line of the C race throughout its history and ranging from slight increases in the rate of pulsation to structural changes involving the production of supernumerary apparatus can only indicate a greatly increased rate of excretion.

d. On the non-contractile excretory vacuoles. Calkins ('04) in describing the degeneration changes in the protoplasm of Paramaecium during depression periods notes the appearance in the endoplasm of large shining vacuoles ('starvation vacuoles' of

Wallengren, '01), which may appear in paramaecia well provided with food and still forming gastric vacuoles. Food in the case of starved Paramaecia, stimulation in the case of those undergoing a depression period, results in the disappearance of these vacuoles.

Vacuoles of this description were characteristic of the thyroid Paramaecia of these experiments, especially at times of rapid division. Sample individuals are shown in figures 9, 10 and 11. Examination of the media at such times showed thyroid and

Fig. 9 Paramaecium caudatum after 220 days thyroid treatment, showing non-contractile excretory vacuoles. Drawn from life.

Fig. 10 Paramaecium caudatum after 220 days thyroid treatment, showing non-contractile excretory vacuoles. Drawn from life.

bacterial food present in abundance. Gastric vacuoles were being formed at the normal rate.

A chance observation may serve to throw some light on the origin of these enormous non-contractile vacuoles. In a Paramaecium under observation it was noticed that the contractile vacuoles had lost their connection with the exterior, although normal in all other respects, swimming about and forming gastric vacuoles. For a considerable time the feeding canals continued normal pulsations, at each of which the vacuole proper became enlarged. Finally the two enlarged vacuoles swung into the center of the cell, presenting all the appearance of the large non-contractile vacuoles characteristic of starved or thyroid fed Paramaecia. My attention was now diverted to the feeding canals,

some of which had become considerably enlarged, while others had disappeared. One canal of each system finally rounded up and began pulsating (apparently emptying its contents to the exterior). Some of the reduced feeding canals were acquired, others formed *de novo* and in a short time all the appearance of a miniature contractile vacuole with a complete feeding system were assumed. A few spasmodic contractions were recorded, during which time the vacuole increased in size, before the form was accidentally destroyed through the evaporation of the drop of water in which I was observing it. I have not yet had the opportunity of repeating this observation, but I am convinced

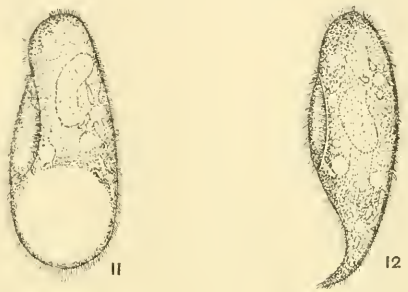

Fig. 11 Paramaecium caudatum after 160 days thyroid treatment, showing non-contractile excretory vacuoles. Drawn from life.

Fig. 12 Same individual shown in figure 11 after expulsion of non-contractile vacuole. Drawn from life.

that this is the mode of formation of the giant non-contractile vacuoles characteristic of my thyroid fed lines.

The fate of these large vacuoles is also of interest. I have repeatedly observed them burst, pouring out their contents to the exterior. No apparent effect was produced upon the Paramaecium except a shrinking of the protoplasm where the large vacuole had been located, resulting in the formation of a tailed or hooked Paramaecium similar to those upon which not a few studies of heredity have been made. The physiological functions of the Paramaecium do not appear to be in the last injured, although the size of the individual be reduced a half by such an explosion. Figure 12 represents the same individual as figure

11 after the expulsion of the large non-contractile vacuole. The malformed individual divided at the same rate as other and more normal thyroid treated sister cells and produced descendants normal (for the thyroid line) in every respect.

These vacuoles then are to be considered another evidence of a greatly increased activity of the excretory system due to the thyroid diet.

e. On monstrosities. From my observations upon the three races of Paramaecia here reported, I have become more and more impressed with the remarkable variability of the Paramaecium morph, and its adaptivity to environmental changes, and by the appearance of apparent "mutations" which have no hereditary significance. The appearance of a Paramaecium with an extra contractile vacuole would at first glance seem almost important enough for the erection of a new species, yet it has arisen in my cultures as the response to an increased amount of excretion produced by thyroid feeding.

The 'hooked' Paramaecium described above was maintained in a sub-culture of thyroid medium where it lost its distinguishing character after a few generations, thereafter, dividing at the same rate as the other thyroid fed individuals and showing no tendency to throw 'hooked' descendants.

A third example may be mentioned. In the A race in one of the thyroid treated lines a form appeared which was almost spherical. Later observations showed that this type of individual was due to a form of division in which the posterior cell was twice as long as the anterior. Seven cases of this asymetrical mode of division are recorded in my notes. Four were established as a sub-culture and treated with thyroid, three were maintained in control medium. The latter died within twenty-four hours. Those in the thyroid medium after a few divisions producing cells like themselves regained the normal shape and after being maintained a few generations longer without throwbacks were abandoned.

These cases are introduced out of many which have occurred in the course of the experiments, in none of which have I seen any evidence of any inheritance of the acquired character or

any tendency toward its repetition. I have also considered the question as to whether the results on division rate might not be considered due to more or less unconscious selection. I believe the constancy of the results belies any such objection, but may add that many attempts to increase or decrease the division rate permanently by selection in my thyroid lines have been unsuccessful.

f. General considerations. There seems to be much evidence in favor of the supposition that the thyroid contains a 'dissimilatory' hormone (Biedl, '13). To this position my results appear to lend themselves. They demonstrate an increased rate of division as an effect of thyroid feeding. Coincident with this a remarkable derangement of the excretory apparatus has been observed. Increase in structure and function are striking evidence of an increase in the amount of excretion. Observations on the rate of formations of the gastric vacuoles show that there is no corresponding increase in the amount of food ingested. If without prejudice as to the nature of the chemical reactions taking place we admit the validity of a distinction between assimilatory and dissimilatory phases of metabolism, these facts may be interpreted as an increase in dissimilatory function produced in the metabolism of Paramaecium by thyroid feeding.

SUMMARY AND CONCLUSIONS

1. Thyroid substance fed to Paramaecium aurelia or caudatum, either as emulsion of raw thyroids or as a suspension of the commercial powder, produces a constant and significant increase of 65 per cent in the rate of division over that observed in the common laboratory medium—hay infusion.

2. The thyroid is the only one of the internally secreting glands that produces this effect.

3. Boiling the thyroid produces no change in the reaction. Iodothyrin and iodine fail to produce the thyroid effect.

4. Paramaecia after prolonged thyroid treatment revert to the normal division rate when returned to the control medium.

5. The life history curves of the thyroid treated lines show the same depression periods at the same time intervals as the

control lines, and the thyroid produces the greatest acceleration of the division rate when the control line is dividing most rapidly.

6. Paramaecia ingest and digest particles of the thyroid.

7. There is no significant difference between the number of gastric vacuoles formed in a given time between the thyroid fed and the control Paramaecia.

8. There are profound disturbances of the excretory system in the thyroid fed Paramaecia. The most remarkable of these is an increase from two to three contractile vacuoles.

9. The thyroid fed Paramaecia manifest at all times a highly vacuolated protoplasm, such as has been described for starved Paramaecia, even during their periods of most rapid division. It has been shown that these large vacuoles are to be considered non-contractile excretory vacuoles.

10. It is suggested that the results of thyroid feeding here noted are due to the presence of a remarkably stable hormone in the thyroid which may be classified among the 'dissimilatory' hormones.

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THE EFFECT OF MEDIA OF DIFFERENT DENSITIES ON THE SHAPE OF AMOEBAE

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NINE FIGURES

For a long time the different species of amoebae were determined by their general shape and by the form of their pseudopodia. Then Scheel showed in 1899 that Amoeba radiosa is not a distinct species but merely an early stage in the life history of Amoeba proteus. The long slender pseudopodia of A. radiosa give place to the lobose ones which are typical of amoeba proteus and become its organs of locomotion.

While working with an amoeba of the limax group I noticed a great variety in the form of the animals, the shape of their pseudopodia, and also in the size of the individuals. It was with a view of determining what the factors are which bring about these differences that the present work was begun.

In an interesting paper on the growth of the epithelial cells of the frog's skin, Uhlenhuth ('15) has shown that when the cells are grown on media of different consistencies there is a distinct difference in their shape. In a firm medium the epithelial cells are polyhedral and remain united in a compact membrane. In the semi-firm medium the cells which stray out are fusiform in shape, while in the soft medium the cells are fusiform or thread-like in shape. With these experiments in mind I determined to try raising amoebae on media of different densities to see whether they too would be affected in the same way as the epithelial cells of the frog skin.

MATERIAL

The material used for these experiments came from a pure line of a limax amoeba. This amoeba had been isolated and the pure line started nearly a year ago. Since then it has been growing on agar-agar made up with tap water and Witte's peptone.

It may be interesting to note the method of isolation used. The amoebae were allowed to spread out on the agar in the Petri dishes until those at the edge were afar apart from one another. Then small blocks of agar containing one amoeba were cut out and placed on fresh culture dishes.

The amoebae were studied in media of six different densities; water, and agar-agar of 0.5, 1, 1.5, 2, and 2.5per cent. To these different preparations of agar, which had been made up with tap water, 0.4 per cent of Witte's peptone was added and the media were then sterilized in the autoclave for twenty minutes under fifteen pounds of pressure. The precaution was taken that all the media should be cooked the same length of time and should in every respect be treated exactly alike so that the relative densities would be the same.

Wherry's ('13) medium of water, egg albumen, and the yolk of the hen's egg was made up in the different percentages of agar. Unfortunately the bacteria on which the amoebae live multiplied so rapidly here, that the amoebae were soon exterminated, though at first they had been unusually large.

The agar of 0.5, 1, 1.5, 2, and 2.5 per cent was poured out into sterile Petri dishes. Agar of 0.5 per cent was also poured upon depression slides, as it was often impossible in warm weather to invert the Petri dish containing the thin agar. These slides were kept in moist chambers. The amoebae were transplanted from old cultures by means of the platinum loop.

In order to make camera drawings pieces of agar containing the amoebae were cut out and put on a slide. In this way a much higher magnification could be obtained than with the inverted Petri dishes. For the drawing of amoebae on 0.5 per cent agar those grown on the depression slides had to be used as it was impossible to cut blocks from this soft medium.

The following work is based on the study of 83 different cultures. All the drawings were made with a camera lucida. A Zeiss No. 12 ocular and a 16 mm. Spencer lens objective were used.

EXPERIMENTS

Agar-agar of 2.5 per cent was very firm and dense. On this medium the amoebae multiplied and spread out over the Petri dishes in an ever-widening circular area of irregular outline. This area was limited by the bacteria on which the amoebae live. As these bacteria multiply and spread over the agar, the amoebae follow them. A few amoebae go out beyond them but the place where the bacteria stop is mostly marked by a dense ring of amoebae eight or ten deep. The amoebae on this medium are large, flat, and have few pseudopodia as they are practically surrounded by their food. Locomotion was very slow. Figure 1 shows such amoebae taken from a two day old 2.5 per cent agar medium.

In striking contrast to these are the amoebae which are grown on the 0.5 per cent agar. Figure 2 shows such amoebae which have been two days on this medium. They are surrounded by bacteria, and are considerably smaller, more elongated, and have small irregular pseudopodia. Figure 3 is of amoebae which have been seven days on 0.5 per cent medium. They show the same characteristics.

The amoebae grown on the 0.5 per cent medium are quite evenly distributed over the Petri dish. They are never massed together as in the cultures of denser media, but wander freely. Occasionally one finds them forming a ring at the edge where the bacterial growth stops. This is never a dense ring. It is seldom more than three or four deep with amoebae. The difference in the shape and size of these amoebae and those grown on the 2.5 per cent agar medium is due partly to the fact that the bacteria are not so plentiful here and the amoebae must wander farther for their food supply. Why the bacteria do not multiply so readily on this medium is a question. There is the same amount of peptone as in the denser media. It must be that the agar itself furnishes some food which in the 0.5 per

cent medium is not sufficient for the great growth and multiplication of the bacteria.

The amoebae grown on the 1, 1.5, and 2 per cent agar media show slight differences in the form and shape of their pseudopodia. Those grown on the 1 per cent agar are similar to those grown on the 0.5 per cent agar, and those on the 2 per cent agar are similar to those grown on the 2.5 per cent agar.

On the 1 per cent agar the amoebae multiplied very slowly and spread out rather evenly over the Petri dishes but soon encysted. Figure 4 shows some amoebae from a week old culture. The pseudopodia are irregular and the animals slightly elongated. This medium was not well suited to the growth of the amoebae.

In figure 5 the amoebae had been one week on the 1.5 per cent agar. The pseudopodia, when present, are very small, irregular protrusions of the ectoplasm. The amoebae are of good size. This is an excellent medium for their growth and multiplication. The 2 per cent medium is an equally good one for their growth. Both here and on the 1.5 per cent agar the bacteria do not multiply more rapidly than the amoebae. In other words the food supply and demand seem to be more evenly balanced. Figure 6 shows amoebae grown on the 2 per cent medium. The pseudo-

Fig. 1 Amoebae drawn from a 2.5 per cent agar medium, two days old.

Fig. 2 Amoebae drawn from a 0.5 per cent medium of agar. Two days old. Surrounded by bacteria.

Fig. 3 Amoebae from a seven day old 0.5 per cent agar culture.

Fig. 4 Amoebae from a seven day old 1 per cent agar medium.

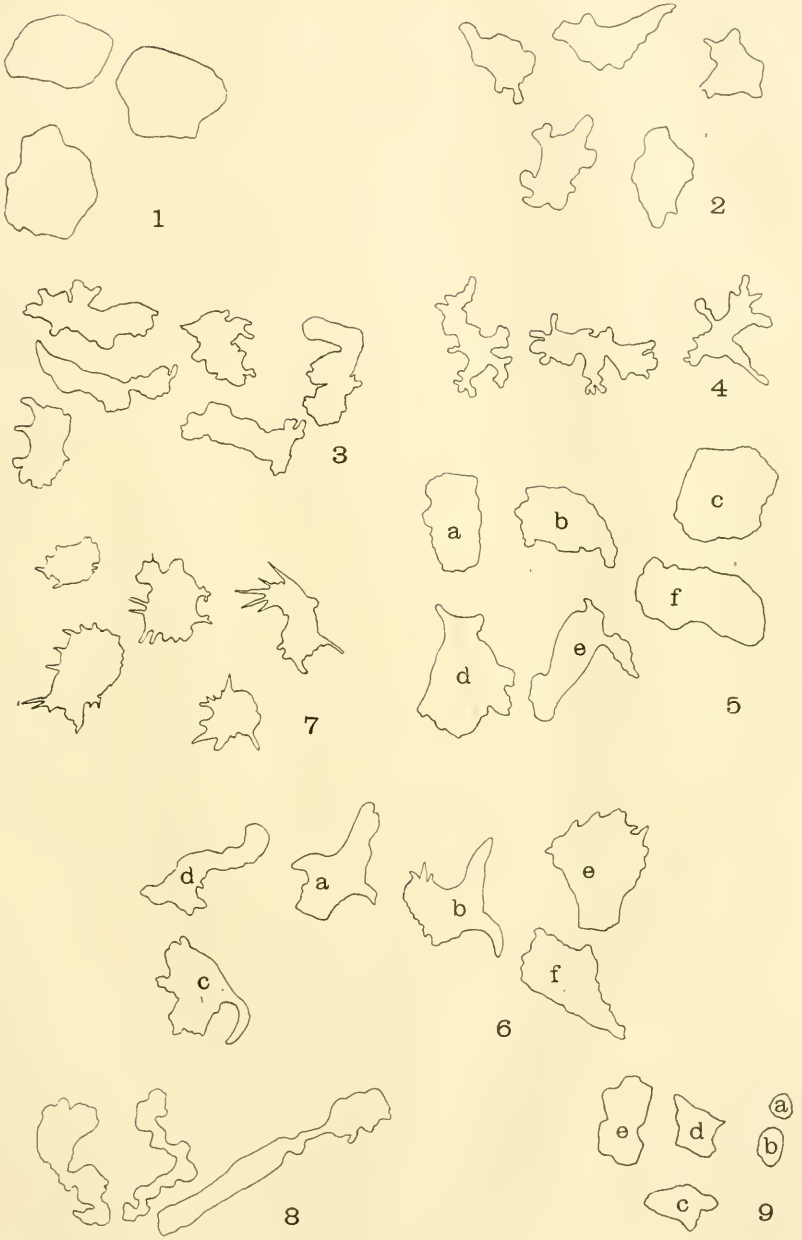
Fig. 5 Amoebae from a 1.5 per cent agar medium. *A*, *b*, and *c*, were surrounded by bacteria. *D* and *e* were beyond the bacterial zone. *F* had a few bacteria around it.

Fig. 6 Amoebae from a seven day old 2 per cent agar medium. *A*, *b*, and *c* had a few bacteria around them. *D* and *f* were beyond the bacteria. *E* was surrounded by bacteria.

Fig. 7 Drawings made of amoebae soon after they were transferred from 1.5 per cent agar medium to water.

Fig. 8 Amoebae surrounded by bacteria. From an old culture.

Fig. 9 *A* and *b* are amoebae from the center of an old culture, showing the small size of the amoebae in these cultures. *C*, *d*, and *e* are from the edge of the culture.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podia are larger and more numerous than in the amoebae grown on 2.5 per cent agar.

When the amoebae of a 1.5 per cent medium are transferred to a drop of tap water they immediately change shape. Slender pointed pseudopodia (fig. 7) are sent out from all sides. These are later withdrawn and a large anterior ectoplasmic pseudopodium formed. With this the animal advances as it did when on agar, only here locomotion is more rapid. The pseudopodia are merely feelers used while the amoebae is floating in the water before settling on the slide or cover-glass. This process has been fully described by Jennings ('04).

From these experiments and drawings it seems evident that the shape of the amoeba is dependent on the density of the medium on which it is grown. Let us compare the work of Burrows and Uhlenhuth with the present work and see whether their explanation of the change in the shape of the growing tissue cells will fit the case of the amoebae.

Burrows ('13) finds that the cells move freely in thin media. He explains this first by "the better oxygen supply to the tissue fragment and migrating cells. The increase in the supply of oxygen to the tissue fragment is associated with an increase of the metabolism of its cells and a greater production of repelling tubstances. Second, the repelling substances diffuse in greater concentration in the thin restricted areas of the medium." As they diffuse through the medium they affect the shape of the cells. Where they are most dense, i.e., near the old tissue, the cells become contracted. As the posterior ends of the cells contract the anterior ends flow out.

Uhlenhuth ('15) has supplemented Burrows' theory by adding that "the cell plasm in flowing out simply obeys the law of gravity and this movement is initiated at the moment when the relation between the consistency of the medium and the consistency of the cell plasm has attained a certain value, not yet ascertained, brought about by the reduction of the firmness of the medium."

In the case of the amoebae grown on a thin medium (0.5 per cent agar) we must have this desired relation between the consistency of the medium and the consistency of the cell plasm.

Here we have the greater freedom of movement and the resulting lengthening out of the amoeba and the formation of pseudopodia. This is even more strikingly shown in the case of the amoebae transferred to water (fig. 7).

On the thicker media the waste products do not diffuse so rapidly and consequently this relation between the cell plasm and medium is not so easily obtained. It is therefore to be expected that we should not find the amoebae here wandering so freely nor having so many pseudopodia. This is the case. We find on the denser media the cells are massed together in great groups. They are surrounded by bacteria, so have plenty of food for growth. They divide and multiply rapidly, with only those at the edge wandering out into the medium. The great accumulation of waste material must liquefy the medium so that those farthest from the mass of amoebae can move freely outward, having here made for themselves the same relation between the cells plasm and the medium as the amoebae on the 0.5 per cent agar medium normally have. When such a culture, which has divided rapidly, is two or three weeks old, depending on the rate of division and the rate of metabolism, we find the amoebae all over it are spindle shaped and elongated (fig. 8) showing that the medium has been sufficiently liquefied by the waste products to allow this freedom of motion. We also note that many of the cells are smaller (fig. 9), which is no doubt due to the lack of oxygen, as suggested by Burrows ('13).

Later, however, when the accumulation of waste products is very great, and the amoebae have multiplied so fast that there is no further place for them to go, they encyst. I have frequently observed a culture dish containing thousands of amoebae, with plenty of bacteria as food, soon become covered with cysts, the amoebae having often encysted over night. When these amoebae are transplanted to a new culture medium they come out of their cysts (Hogue '14) and are soon multiplying and spreading over the new medium, where the oxygen supply is plentiful and where the waste products have not yet accumulated.

SUMMARY

1. An amoeba limax was grown on agar media of different densities, made up with Witte's peptone.

2. On 2.5 per cent agar the amoebae are rather circular, with few pseudopodia. Compared with those grown on the thin medium they are more numerous and often massed together.

3. On 0.5 per cent agar the amoebae are elongated, with irregular pseudopodia. They move freely on this medium and are evenly distributed over it.

4. On 1, 1.5, and 2 per cent agar the amoebae show the forms intermediate between the amoebae grown on 0.5 and 2.5 per cent agar respectively.

5. The media best adapted to the growth and multiplication of the amoebae are 1.5 and 2 per cent agar plus 0.4 per cent Witte's peptone.

Wellesley, Mass.,
October 4, 1916.

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THE EFFECT OF X-RAYS ON THE LENGTH OF LIFE OF TRIBOLIUM CONFUSUM

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FIVE FIGURES

INTRODUCTION

A great deal of work is reported in the literature on the effect of X-rays on various forms of animal life. A study of this literature shows that, interesting though the results may be, it is with few exceptions, difficult to duplicate the experiments because the physical data relating to the dosage have been so incompletely given. Excluding work on human beings, the following work may be mentioned.

Hastings, Beckton and Wedd[1] found that the hatching of silkworm eggs was accelerated by X-rays (dose not given) and that the second generation was less fertile. Bordier[2] X-rayed six silkworms, giving them a dose of 7 to 8 H,[3] at some unknown penetration. He found an increased restlessness and smaller size. The cocoon was only half size, and the moth did not emerge. Hasebroeck[4] was able to kill caterpillars of Charaxes (dose not given), but those of Vanessa urticae after being X-rayed an unknown amount developed into butterflies which were unable

[1] Hastings, Beckton and Wedd, Arch. Middlesex Hosp., 11th Cancer Report, 1912.

[2] Bordier, Le Radium, 2 p. 410, 1905.

[3] Holzknecht units are measured by means of the change in color produced by X-rays in a pastille of Barium Platino-cyanide. The reading of these pastilles varies considerably with the wave-length of X-rays used so that the X-ray measurements made by such pastilles are meaningless except when the voltage across the tube is given, or when the 'penetration' of the rays is given in some other reliable way. A better method of measuring X-rays is given later in this article.

[4] Hasebroeck, Fortschr. a.d. Geb. der Roent., 11, p. 53.

to fly. Lopriori[5] found a destructive action on Vallisneria spiralis, Genista and Darlingtonia (dose not given).

Perthes[6] X-rayed the ova of Ascaris megalocephala giving them a dose of 24 H at a voltage corresponding to 6.5 cm. spark gap. Cell-division was so much retarded that although the control specimens were in the 4-cell stage at the end of 36 hours, the rayed specimens were still in the 1-cell and 2-cell stage. Hastings[7] rayed eggs of the same species, using an unknown quantity of characteristic X-rays from copper. He found a retardation in growth. Runner[8] X-rayed the eggs of the cigarette beetle (Lasioderma serricorne) using a Coolidge tube, and giving a dose of 150 milliampere-minutes at 65 kilovolts at a distance of 7.5 inches. He found that eggs less than 3 days old failed to hatch, and became shrunken in about 10 days. Eggs over 3 days old hatched but the larvae never reached the pupa stage. Larvae similarly rayed refused to eat, and although they lived a long time after raying, they never reached the pupa stage.

Gilman and Baetjer[9] found that X-rays (amount not given) accelerated the development of eggs of Amblystoma for about 10 days, but produced monsters. They also rayed hens' eggs (dose not given) and found an accelerated development for 36 hours with the final production of monsters. Bordier and Galimard[10] gave incubating hens' eggs daily doses of X-rays of 15 H each (penetration not given) for 20 days. The eggs contained no embryos. In specimens which had been allowed to develop somewhat before raying, growth was arrested at the first dose. These results were confirmed by Gaskell[11] but he makes no record of dose.

Lengfellner[12] X-rayed three pregnant guinea pigs, three days before term (dose not given). Two of the mothers were at

[5] Lopriori, cit. Schaudin in Pflueger's Arch., 77, p. 31, 1899.
[6] Perthes, Deut. med. Woch., 30, 1904.
[7] Hastings, Arch. Middlesex Hosp., 11th Cancer Report, 1912.
[8] Runner, Jour. of Agr. Research, June 12, 1916.
[9] Gilman and Baetjer, Am. Jour. Physiol., 10, p. 222, 1904.
[10] Bordier and Galimard, Jour. d'Elect. Med., p. 491, 1905.
[11] Gaskell, Proc. Roy. Soc., B 83, February 28, 1911.
[12] Lengfellner, Munch. med. Woch., p. 44, 1906.

once killed. Their young died in 10 minutes. The third mother had a miscarriage in 5 hours, the young were all dead. He also rayed one hind leg of an 8 days old puppy (dose not given). Seven and one-half months later, this leg was 8 cm. shorter than the other. Cohn[13] rayed the heads of pregnant rabbits (dose not given) enclosing the rest of the rabbit in a lead box. Pregnancy continued to full term. For 14 days after birth the young seemed normal, but afterward they became stunted so that after 7 weeks they were only one-third the size of the controls. Försterling[14] found that if he rayed the heads of 40-hour old rabbits (dose not given) the whole animal was stunted, but if any other part of the animal were rayed, only that part was stunted. Krukenberg[15] found that if the pelvis of a young dog or goat is X-rayed (dose not given) the growth of the hind legs was retarded. Raying the shoulders caused ataxia and nervousness, affected the eyesight and made the animal more irritable.

The work showing the possibility of a stimulating effect on eggs is confirmed in an interesting way by studies on single types of cells in animals. Menetrier and Mallet[16] and Rowntree[17] have shown by raying the ears and tails of rats that, somewhere between zero dose and that dose necessary to produce dermatitis, there is a dose which stimulates the growth of epithelial tissue. Benjamin, Reuss, Sleuka and Schwartz,[18] Aubertin and Beaujard,[19] and Murphy and Norton[20] have shown that X-rays in the proper amount may increase the number of leukocytes.

All the above work may be summarized as follows: X-rays may act upon an organism (or on a single type of cell in that organism) in one of three ways: (1) to produce a stimulation; (2) to produce a destructive effect which takes place only after a

[13] Cohn, Verh. d. deutsch. Roent. Gesel., Bd. 2, p. 128.

[14] Försterling, Verh. d. deutsch. Roent. Gesel., Bd. 3, p. 126.

[15] Krukenberg, Verh. d. deutsch. Roent. Gesel., Bd. 5, p. 68.

[16] Menetrier and Mallet, Bull. de l'Ass. fran. pour l'etude du Cancer, 11, p. 150, 1907.

[17] Rowntree, Arch. Middlesex Hosp., Cancer Reports, 1908–1909.

[18] Benjamin, Reuss, Sleuka, and Schwartz, Wien klin. Woch., 19, p. 788, 1906.

[19] Aubertin and Beaujard, Arch. de Med. Exper. et d'Anat. Path., 2, p. 273, 1908.

[20] Murphy and Norton, Science, December 10, 1915.

certain latent interval; (3) to produce an instant destructive effect.[21]

By analogy with the action of various drugs, one would expect that the rays could be made to act in any one of these three ways at will by merely varying the size of the dose. Not a sufficient number of the authors cited above have adequately recorded the dose to enable one to verify this analogy without further experimentation. It is the purpose of this article to record the results of experiments made toward this end.

About a year ago the writer was engaged in some preliminary work on the lethal effect of X-rays on Tribolium confusum. These little beetles are ordinarily called "flour weevils" and are said by Chittenden[22] to be the most injurious enemy to prepared cereal foods. In two years from the time of their recognition as a distinct species they had spread to nearly every state in the Union, and even as early as 1895 are said to have cost the millers of the United States over $100,000 in manufactured products alone. It was found possible to destroy the eggs of these beetles with X-rays, thus giving hope of a new technical use for X-rays, but the most interesting results from a scientific point of view were obtained from the beetles themselves.

PRELIMINARY EXPERIMENTS

It was found that these beetles could be killed with X-rays if a sufficiently large dose were given, but it was noticed that the beetles did not die for several days after they were rayed. Further experiments indicated that the length of this latent interval depended upon the amount of the X-ray dose, and there seemed to be some evidence that this relation was approximately logarithmic. It was therefore decided to repeat these experiments more carefully, first making sure that the effect was really due to X-rays and not some attendant circumstance, and then in-

[21] A full bibliography of work done up to 1912 may be found in Fortschr. a.d. Gebiete der Roent., 19, p. 123, 1912, in an article by Walter. A complete bibliography of all X-ray work since 1912 has been published by Gocht.

[22] Chittenden, Bull. No. 4, New Series, Revised Ed. U. S. Dept. of Agr., p. 113, 1902.

vestigating the relation between the latent-interval and the X-ray dosage. This required a large number of beetles and it was necessary to determine their life history and how best to propagate them. It was found that they grow and propagate best in oatmeal or whole-wheat flour, but they will live in corn meal, white flour or any of the prepared cereal products. Propagation takes place best at a temperature of 35 to 36°C. and at high humidity. Temperatures of 45°C. or over are fatal. The eggs are white and from 0.3 to 0.6 mm. in diameter. They are usually associated with pieces of grain. Larvae grow to a length of 5 or 6 mm. and shed their skins six times. Pupae are white. Young beetles are a light straw color which later darkens to a russet. The beetles are about 4 mm. long. They are especially adapted to such work as is reported here because they are small, harmless, easy to handle and count in large numbers; they propagate readily, can not crawl out of glass beakers or small porcelain crucibles, and show little tendency to fly.

In the preliminary experiments mentioned above, the beetles were packed in small wooden pill boxes with some food. There were 25 beetles in each box. There was a possibility that death was not due to any action of X-rays on the beetles, but might have occurred from any of the following causes:

1) Lack of air and food
2) High temperature due to overcrowding
3) Injury due to overcrowding
4) NO_2 caused by the high voltage connections of the X-ray tube.
5) Ionized air
6) Excessive humidity
7) Effect of X-rays on the food in the boxes or even on the boxes alone.

The following seven experiments were therefore done.

Experiment 1. To show tha' the beetles were not killed by lack of air and food rather than by X-rays.

A. 10 beetles were sealed up in a glass tube with a rubber stopper, without food, in a space 0.05 cubic inch. They were all alive at the end of 76 hours.

B. 20 beetles were similarly sealed up, without food, in a space 0.05 cubic inch. They were all alive at the end of 3 weeks, but some of them seemed to be stuck together by a film of moisture. At the end of 4 weeks, 8 were still alive.

C. 10 beetles were similarly sealed up in a space 0.05 cubic inches together with a pinch of white flour. They were all alive at the end of 8 weeks. Evidently the flour had taken care of the bodily moisture noted in B.

Therefore, Tribolium Confusum are not easily killed by lack of air, provided they are kept dry and have food.

Experiment 2. To show that the beetles were not killed by some effect of temperature, rather than by X-rays.

20 beetles were placed in a round-bottom test tube, $\frac{3}{8}$ of an inch in diameter, well heat-insulated. Temperature was measured from time to time by means of a delicate thermocouple. The highest temperature reached was 27.25°C.

Therefore, the beetles can not by over-crowding in a heat-insulated space raise their temperature high enough to produce death.

Experiment 3. To show that the beetles were not killed by mechanical injury rather than by X-rays.

A. 5 beetles were placed in a tapered test tube. Diameter of test tube was $\frac{3}{8}$ inch. Length of taper was $1\frac{1}{8}$ inch. A small hole in the bottom gave ventilation. The beetles were therefore crowded together, and repeatedly crawled over one another in their efforts to escape. All were alive at the end of 97 hours.

B. 5 beetles were shaken violently in a glass beaker 50 times a day. At the end of two days, 4 were still alive. At the end of a week, 3 were still alive.

C. In addition, it may be stated that beetles used as 'controls' in the main body of this work do not seem to be at all affected by being dropped through a funnel several times daily during the process of counting.

Therefore, the beetles are not easily given fatal injuries.

Experiment 4. To show that the beetles were not killed by NO_2 rather than by X-rays.

A. 20 beetles were put in a test tube $\frac{3}{8}$ inch in diameter with a little corn meal. The test tube was connected to a source of NO_2. All beetles were alive after having been in an atmosphere of NO_2 for 25 hours. At the end of 64 hours, 5 were alive.

B. 25 beetles were put in a vial and exposed to dilute NO_2 for 5 minutes. The concentration of NO_2 was such as to distinctly color starch-KI paper in $1\frac{1}{2}$ minutes. 23 beetles were found alive after the 20th day. But there is not enough ozone and NO_2 together in the lead box where the beetles are X-rayed to color starch-KI paper during an exposure of 14,000 $\frac{MAM}{25^2}$ at 50 KV.

Therefore, there is not enough NO_2 produced while the beetles are being X-rayed to affect them.

Experiment 5. To show that the beetles were not killed by ionized air rather than by X-rays.

25 beetles were carefully shielded from X-rays. Ionized air, together with what little ozone and NO_2 might be present was drawn past the beetles during an exposure of 19,600 $\frac{MAM}{25^2}$ at 50 KV. Even if only 10 per cent of the ions remained uncombined when they reached the beetles, still this would be equal to that caused directly by 1960 $\frac{MAM}{25^2}$ at 50 KV. This dose of X-rays, acting directly on the beetles, would have killed them all in less than 2 weeks if death were produced by ionized air rather than by X-rays directly. But at the end of 21 days, only 1 beetle was dead.

Therefore, the beetles are not killed by ionized air.

Experiment 6. To show that the beetles were not killed by too high humidity rather than by X-rays.

A. 10 beetles were put, without food, in a flat-bottomed test tube $\frac{3}{8}$ inch in diameter, which was kept dry by a side tube filled with P_2O_5. All but one were alive after 6 days.

B. 60 beetles were gathered in such a way that each beetle was slightly moistened on the back. They were all put, without food, in a test tube, 1 inch in diameter. As they crawled over each other, the moisture was spread over their whole bodies. In 6 hours most of the beetles were dead. Those alive were so weak that they could not turn over, even when lying on their sides. 60 other beetles gathered in the same way, and put in a similar test tube, with a cloth bottom, lived. The cloth bottom could only have acted as a ventilator and an absorber of water.

C. Beetles are grown in an almost water-saturated atmosphere in the brooders and seem to thrive well.

Therefore, the beetles are not harmed by either extreme dryness or by high humidity, but may be killed by strangulation when water is condensed on them.

Experiment 7. To find effect of X-rays on the food of the beetles.

A box similar to those used in the preliminary experiments was filled with corn meal and X-rayed 15,000 milliampere-minutes at 25 cm. distance at 50 kilovolts. 25 beetles were then put in this box with the cornmeal. They lived lives of normal length. But beetles rayed this amount die almost instantly.

Therefore, X-raying the boxes and the food has no effect upon the length of life of the beetles.

In the light of the above experiments, it seems safe to conclude that the death of the beetles recorded below was due to X-rays, rather than to some accidental circumstance.

APPARATUS

X-rays were produced by a water-cooled Coolidge tube (tungsten target) operating directly from a high-tension 60 cycle transformer. Such tubes will rectify their own current up to 50 to 100 milliamperes, at 50 kilovolts (R. M. S.).[23] Oscillograph tests showed that the transformer was of such a type that when operated under the above conditions, the wave-form of the secondary (high voltage) resembled that of the primary (low voltage), and the inverse voltage did not exceed the direct voltage (i.e., operating voltage of the tube) by 5 per cent. Further tests with the oscillograph showed that the R. M. S. voltage of the secondary differed from that shown by a voltmeter coil by not more than 3 per cent. Tube voltage was therefore measured in terms of R. M. S. kilovolts, as shown by the meter.

The voltage impressed upon the primary was controlled by means of an auto-transformer of such size as to cause no appreciable change in wave-form. The wave-form used was very nearly sinusoidal.

The filament of the X-ray tube was heated by current from a small transformer. This was connected through a ballast transformer to the terminals of the circuit supplying the auto-transformer. Connections are shown in figure 1.

Tube current was measured with a direct-current milliammeter. Since this current was pulsating (half of every wave being suppressed by the rectifying action of the X-ray tube), oscillograph records were taken to compare the meter-reading with the instantaneous value of the current. It was found in all cases that the value of the mean current as shown on the meter was almost exactly half the value of the peak of the wave. The current through the tube was therefore read in mean milliamperes on the meter. The wave-form of this current was similar in every way to that of other Coolidge tubes.

The X-ray tube was in a lead box whose walls were $\frac{1}{4}$ inch thick. This provided a safe protection from X-rays at the volt-

[23] R. M. S. voltage is measured as the "root mean square" of the voltage wave. This is in accordance with ordinary electrical practice in measuring alternating voltages.

ages used. The tube was connected to the transformer by $\frac{1}{2}$ inch rods, to prevent corona. Where these rods entered the lead box, the lead was replaced by lead glass 1 inch thick, which acted both as insulation and as X-ray protection.

A chamber of lead (*A*, fig. 1) 7 cm. square and 5 cm. long was placed in the wall of this lead box, directly opposite the focal spot of the X-ray tube. A sheet of aluminum 0.025 mm. thick was fastened across the end of the chamber nearest the X-ray tube. This protected the interior of the chamber from electrostatic effects, and prevented any NO_2, ozone, etc., from the in-

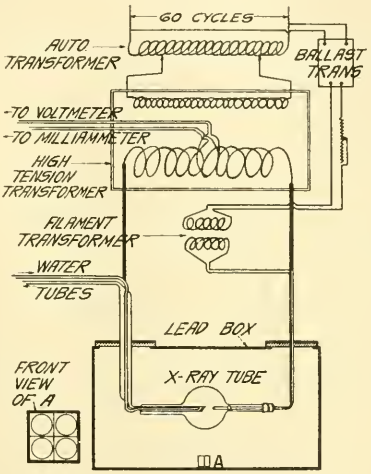

Fig. 1 Diagram of apparatus

terior of the lead box, and any radiant heat from the X-ray tube from entering the chamber. The lead sides of the chamber protected the interior from any secondary X-rays which might be produced on the walls of the lead box. The only rays which could enter the chamber were those sent out directly from the X-ray tube itself.

Into this chamber were placed, four at a time, the boxes of beetles to be rayed. These boxes were of wood, cylindrical in shape, $1\frac{1}{8}$ inches in diameter and $\frac{5}{8}$ of an inch high. The wood was $\frac{1}{8}$ of an inch thick. Each box contained 25 beetles and a

little cornmeal, and was kept closed during the raying. The X-rays, therefore, after leaving the X-ray tube, passed through 0.025 mm. of Al and 3 mm. of wood before reaching the beetles and cornmeal. At the voltage employed in this work to date (50 KV_{RMS}), the error due to absorption of X-rays by the small thickness of Al and wood was very small.

EXPERIMENTAL

Two or three thousand beetles were gathered from the same brooder on the same day and put into a large granite-ware pail. The next morning they were packed with a little sterile cornmeal in the wooden boxes mentioned above,—25 in each box. In this way the distribution of age, susceptibility to X-rays, etc., was as nearly uniform as possible. From this time on the tightly closed boxes were kept in incubators at 35° to 36°C. and at saturated humidity, except while being X-rayed or while being counted. Every box was opened daily, the beetles separated from the cornmeal and a record made of the number of live and dead beetles. The assistants who did this counting had no way of knowing the dose of X-rays which had been given.

After all the beetles in a given group of boxes were dead, the data sheets were collected and the data combined as shown in table 1. From 4 to 8 control boxes were used with each experiment to make sure that the beetles were in every way normal. The normal death rate at the end of the first 15 days was

TABLE 1

Beetles rayed $2000\frac{MAM}{25^2}$ *at 50 KV*

BOX	DAYS									
	0	1	2	3.2	4.2	4.8	5.2	6	7	7.2
36	0	0	1	1	5	14	17	22	24	25
37	0	0	0	0	6	15	19	24	25	25
38	0	0	0	0	5	16	18	24	25	25
39	0	0	1	1	9	15	18	24	25	25
Total dead...	0	0	2	2	25	60	72	94	99	100
Per cent dead	0	0	2	2	25	60	72	94	99	100

never more than 4 per cent. Beetles rayed 500 $\frac{MAM}{25^2}$ at 50 KV. were practically all dead in 15 days. Beetles rayed larger doses were all dead in less than 15 days. Therefore no correction for normal death rate of the X-rayed beetles was considered necessary.

It was found that while all the beetles in a given box did not die at the same moment, there was very narrow range of time during which most of them died, thus suggesting that we were dealing with a quantitative effect which could be studied to some good. For example, the results given more in detail in tables 1 and 2 and in figure 2, show that if the dose was 15,500 $\frac{MAM}{25^2}$ at 50 KV. all the beetles were dead at the end of the raying; if the dose was 2000 $\frac{MAM}{25^2}$ at 50 KV. practically all the beetles died between the third and the sixth days after raying, while half of them died between the fourth and fifth days; if the dose was 500 $\frac{MAM}{25^2}$ at 50 KV. death took place between the fourth and the ninth days, while half of them died between the sixth and eighth days. Doses less than 500 $\frac{MAM}{25^2}$ at 50 KV. were not fatal to all the beetles.

Now if the percentage of dead beetles is plotted against the time which has elapsed since they were X-rayed, it is evident

TABLE 2

Beetles rayed 500 $\frac{MAM}{25^2}$ *at 50 KV*$_{RMS}$

BOX	DAY												
	0	1	2	3	4	5	6	7	8	8.3	9	10	11
23	0	0	1	1	1	2	11	18	24	24	24	25	25
24	0	0	0	0	0	1	5	9	17	20	23	24	25
25	0	0	0	0	1	3	6	19	22	24	25	25	25
26	0	0	1	1	1	1	6	11	20	23	25	25	25
Total dead.....	0	0	2	2	3	7	28	57	83	91	97	99	100
Per cent dead.....	0	0	2	2	3	7	28	57	83	91	97	99	100

that the points follow a smooth curve, which is the integral of a probability curve. If now the slope of this curve is plotted against its abscissae, a probability curve may be obtained. If the beetles represented by two such curves have been gathered from the same brooder at the same time, the corresponding points on the two curves may be compared, for they represent beetles of corresponding resistance to the action of the X-rays. It will be noticed that the curve approaches the zero and the 100 per cent lines asymptotically. This is in agreement with the well known fact in toxicology that some individuals are expecially susceptible to a given harmful agent, so that a very small dose causes death, while other individuals are especially resistant to the same agent so that they continue to live for a comparatively long time, even when given large doses. The steepness of the curves as plotted in figure 2a is a measure of the idiosyncrasy.

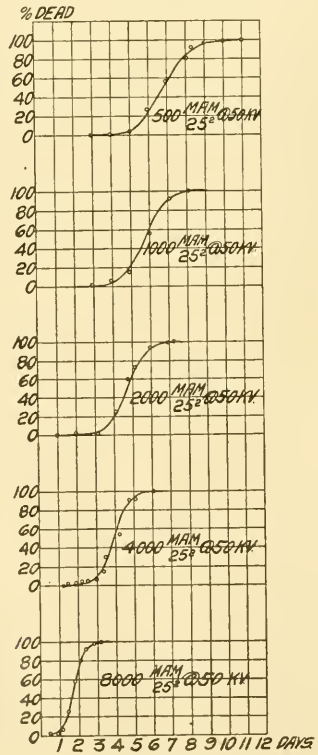

Figure 2*A*

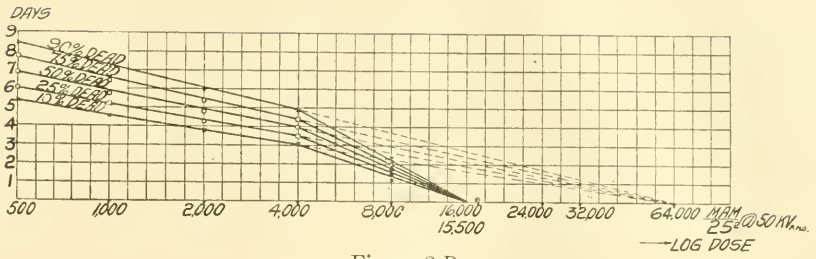

Figure 2 *B*

Fig. 2 *A*, curves showing percentage of beetles dying each day after raying. *B*, days-life plotted against the logarithm of the X-ray dose.

This gives a method of handling the data which not only eliminated errors due to idiosyncrasy, but which even gives a measure of idiosyncrasy which is accurate enough between the limits of '25 per cent dead' and 75 per cent dead. Figure 2 *A* shows a series of results obtained in this way in which even the 10 per cent and 90 per cent points could be used. Before discussing figure 2 *B*, it will be necessary to explain the methods of recording X-ray dosage.

In order to define the quantity of X-rays and the bundle of wave-lengths used in a given experiment, it is necessary to record explicitly

1) the material used as a target in the X-ray tube.
2) the thickness and kind of filters (if any).
3) the form of the voltage wave.
4) the form of the current wave.

The following must be recorded either explicitly or implicitly:

5) voltage across the X-ray tube.
6) current through the tube.
7) the length of time the X-rays were employed.
8) The distance from the focal spot of the X-ray tube to the point to be rayed.

If 1, 2, 3, 4 are kept constant throughout the experiment, they may be stated once for all (as was done in this report under the head of 'apparatus'), and the dose of X-rays may then be defined by either of two methods:

a) The voltage may be expressed directly, or an approximation may be given in terms of the readings of a Benoist pene-

trometer or in terms of the Christen "Half Value layer." The other factors may be given in terms of the reading of a Kienböck strip or a Holzknecht pastille, etc.; or better,

b) The voltage and distance are given directly and the product of the current and time is given, thus,

"100 milliampere-minutes at 25 cm. distance at 50 kilovolts." This is usually contracted to read

$$100 \frac{\text{MAM}}{25^2} \text{ at 50 KV.}$$

It will be noticed that the distance is expressed in terms of its square. This is because the intensity of X-rays varies inversely as the square of the distance. Too much stress can not be laid upon the necessity for recording the voltage, and for keeping the voltage-reading constant, for not only does the penetrating power of the X-rays depend upon the voltage, but even the quantity of rays given off by the tube per milliampere depends very largely upon the voltage.

In figure 2 *B*, 'days life' is plotted against the logarithm of the X-ray dose. The 10 per cent, 25 per cent, 50 per cent, 75 per cent and 90 per cent points of the curves for 500, 1000, 2000 and 4000 KV. lie on a family of straight lines $Y = A - B \log. X$ where X is the X-ray dose and Y is the number of days life after raying. All these lines meet the zero line at the point (64,000). The points for 8000 and 15,500 $\frac{\text{MAM}}{25^2}$ at 50 KV. do not lie on these lines, but when taken along with the points for 4000 $\frac{\text{MAM}}{25^2}$ at 50 KV., they are found to form a new family of curves of the same type as the first, but with a steeper slope. The interpretation of this is given later. It should be noted here, however, that beetles rayed more than 4000 $\frac{\text{MAM}}{25^2}$ at 50 KV. were unable to move their legs and antennae easily, but that this effect was not noticed in beetles rayed less than this amount. For this reason it was difficult at the higher dosages to obtain data as accurate as that obtained at the lower dosages.

Curves like figure 2 have been obtained time after time, the only difference being in the height of the ordinates and the slope of the family of curves. Figure 3 shows a typical curve of this sort. It will be noticed that in spite of the difference in the ordinates, the sharp break in the curve occurs at the same dosages

At 50 kilovolts the lowest dose of X-rays which is fatal to all the beetles in 500 $\frac{\text{MAM.}}{25^2}$. In order to explore the field below this dose, 1100 beetles were gathered from the same brooder at the same time and packed into boxes of 25 each with sterile corn-

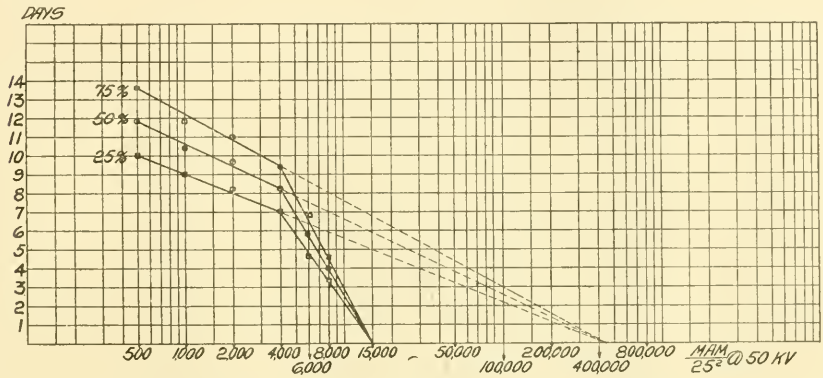

Fig. 3 A typical curve showing days-life plotted against the logarithm of the X-ray dose at 50 KV_{RMS}.

meal, and these boxes were divided into 7 groups of 8 boxes each, and one group of 4 boxes.

One group of 8 was kept as a control. The others were rayed 100, 200, 250, 300 $\frac{\text{MAM}}{25^2}$ at 50 KV. respectively. The group of 4 was rayed 500 $\frac{\text{MAM}}{25^2}$ at 50 KV. The results are plotted in figure 4, curves *A*, *B*, *C*, *D*, *E*, *F*, *G*.

It will be noticed that there is but little difference between curves *A* and *B*. Except for a small hump between 0 and 10 days, there is but little difference between curves *A* and *C*. This is brought out in curve *G* which is the same as curve *C*,

except that the calculations are based on the supposition that there were no beetles dead on the tenth day. This similarity is still more marked between curves *B* and *G*. Except for a similar hump between 0 and 12 days, curve *D* resembles curves *A* and

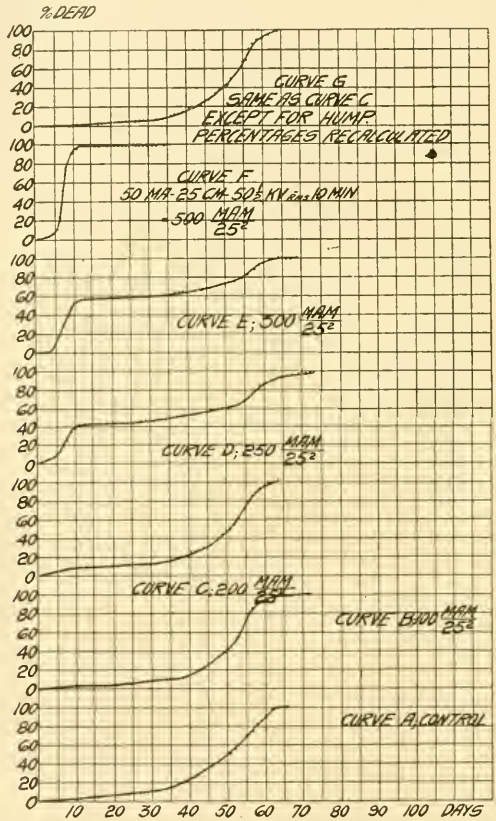

Fig. 4 A series of curves showing the effect of X-ray doses smaller than the threshhold dose.

B. The hump is, however, much higher than in curve *C*. In curve *E* the hump is still of the same shape as in curves *C* and *D* (it is the integral of a probability curve) and covers a period of 12 days, but is considerably higher. In curve *F* the 'hump' is the whole curve, except for a very flat portion which represents a single very resistant beetle.

These curves have been duplicated several times, and although beetles gathered from different brooders at different times give curves of slightly different shape, still all the curves agree very closely with the typical ones shown in figure 4, especially with regard to the 'hump.'

This would make it seem that at 50 kilovolts, 200 $\frac{\text{MAM}}{25^2}$ is the minimum lethal dose of X-rays for the least resistant beetles, and that 500 $\frac{\text{MAM}}{25^2}$ is the minimum lethal dose for the most resistant beetles. The fact that the 'hump' is always of the

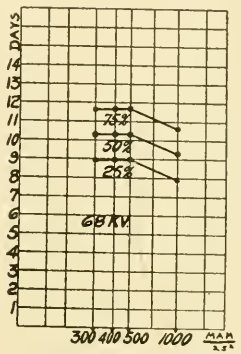

Fig. 5 Days-life plotted against the logarithm of the X-ray dose at 68 KV_{RMS}.

same form suggests that these beetles which would live 9 days with a dose of 200 $\frac{\text{MAM}}{25^2}$ at 50 KV. would live a shorter time if rayed 250 $\frac{\text{MAM}}{25^2}$ at 50 KV., and that some more slightly resistant beetles which would be unaffected by a dose of 200 are killed off at the end of 9 days by a dose of 250. But when a dose of 500 $\frac{\text{MAM}}{25^2}$ at 50 KV. is reached, the most resistant beetles are also affected by the rays, so that the whole graph then approximates the probability integral. Further discussion of figure 4 is reserved for a later paper.

Due to a breakdown of the transformer, the data to date at any other voltage than 50 KV. are fragmentary. Figure 5 shows the data obtained at 68 KV RMS just before the transformer broke down. The beetles were gathered at the same time as those of figure 3 and the raying was done within 3 days of that of figure 3. The two graphs may therefore be compared for what they are worth. It is hoped later to determine more accurately the effect of voltage.

THEORETICAL

It has been shown above that if the dosage of X-rays is sufficiently large, the experimental relation between length of life (Y) and X-ray dosage (X) is of the form

$$Y = A - B \log. X$$

This formula may be easily derived from an extension of the Psycho-physic law which states that a change in response to an external stimulus is directly proportional to the change in the stimulus, but inversely proportional to the amount of the stimulus. (Thus, the flicker-sensation caused by suddenly dimming a light is directly proportional to the amount of dimming, but inversely proportional to the total intensity of the light.) Now let us suppose that the same principle applies to the action of X-rays on living cells.

Let Y = the number of days a beetle will live after being X-rayed.

Let X = the amount of the X-ray dose.

Then d Y is directly proportional to d X and inversely proportional to X. Moreover, an increase in X produces a decrease in Y.

Therefore, $d\ Y = -B \frac{dX.}{X}$

Integrating $-Y = A - B\ (\log. X)$

which is the same as the equation of the experimental graph.

The constant of integration A has at present only a theoretical meaning, for it represents the number of days a beetle would live if it were X-rayed only 1 $\frac{MAM}{25^2}$ at 50 KV. and if no proc-

ess of repair went on inside the beetle, and if there were no other cause of death present.

It will be noticed that this formula takes no account of any cause of death other than X-rays, nor of any process of repair which may go on inside the beetle. It therefore applies only when the X-ray dose has been large enough to completely destroy the protective mechanism of the beetle, and when the damage caused by the X-rays is large enough so that all other causes of death may be neglected. The minimum dose for which this is true is $500 \frac{MAM}{25^2}$ at 50 KV.

It should be noted that length of life after raying is a measure of the resistance of an organism to X-rays, not of its susceptibility. If X-rays are able to kill the organism in two different ways, as by attacking two different kinds of cells, or two different organs, then the experimental graph should be the resultant of two straight lines, but in such a case it must be remembered that susceptibilities not resistances are to be added.

The graphs shown above seem to indicate that Tribolium confusum are affected in two ways by the X-rays, the threshold dose being, for the first way, $500 \frac{MAM}{25^2}$ at 50 KV., and for the second way about $4000 \frac{MAM}{25^2}$ at 50 KV. These graphs would also seem to indicate that the cause of death for dosages between 500 and 4000 is negligible in the presence of that for dosages above $4000 \frac{MAM}{25^2}$ at 50 KV.

SUMMARY

1. It has been shown that the lethal effect noticed on Tribolium confusum beetles after X-raying is really due to X-rays and not to some accidental circumstance.

2. A method has been developed which eliminates the error due to idiosyncrasy, thus making it possible to obtain bio-physical data of a considerable degree of precision.

3. It has been shown that the lethal effect of X-rays on Tribolium confusum bears a definite mathematical relation to the logarithm of the total X-ray dose.

4. An extension of the Psycho-physic law gives a theoretical explanation of the experimental data, if the resistance rather than the susceptibility of the organism to the X-rays is considered.

The effects produced by changes in voltage, and by dividing the dose of X-rays into small parts, will be taken up in a later paper.

A FURTHER CONTRIBUTION TO THE THEORY OF SEX

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FIFTY-THREE FIGURES

In a series of papers published during the last five years we have dealt with the interesting phenomenon of experimental intersexuality, produced by crossing different geographic varieties of the gypsy-moth, Lymantria dispar L. Many of the facts, as well as their theoretical explanation, have been summarized in a recent paper in the American Naturalist.[1] As it will probably be some time before a monographic account of this work, containing all the experimental details, can be published, we wish in this paper to communicate some more facts as well as the theoretical considerations to which our work has led.

I

We must briefly remind the reader of the principal facts and their explanation. It was shown that by crossing different European and Japanese races of gypsy-moths, very different results can be obtained. Some crosses yield normal offspring, but in others the individuals of one sex assume, to a certain extent, the characters of the other sex—they become intersexual. This result proved to be a constant one for definite combinations. Further, the degree of intersexuality was constant in a given cross, but could vary, in different crosses, throughout the entire range of possibilities between maleness and femaleness, the extremes being, of course, the complete transformation of all females into males or vice versa. This transformation occurred in regard to secondary as well as to primary sex-characters.

[1] Experimental intersexuality and the sex-problem. Amer. Natur., v. 50. 1916.

But when a cross resulted in intersexual offspring the reciprocal cross was normal. It was shown, further, that in F_2 generations of such crosses intersexuality segregates, and in such segregation there are definite rules for the appearance of male and female intersexuality.

From these facts the following explanation was derived. Each sex contains the factors for both sexes. Which factors become potent depends upon the quantitative relation of the two sets of factors. Both of them possess a quantitatively definite strength of action or potency. In normal sex-distribution the right combinations are regulated by the heterozygosis–homozygosis mechanism in the following way. In the case of female heterozygosis, the female formula is [FF]Mm and the male formula [FF]MM. Given a definite potency for these factors, it means that the quantitative value of [FF] is higher than one but lower than twoM. Intersexuality was thus explained by the assumption that different races differ in regard to the absolute potencies of these factors. Crossbreeding results, then, in abnormal combinations, giving, for example, to the factor M within the female formula a higher value than that of the F-set. To make this assumption clear we proposed to assume definite values for these potencies. A female is then produced (assuming female heterozygosis) when FF–M is larger than a definite minimum value, e, and a male when MM–FF is larger than the value e. If we give to e the value of 20 of the supposed potency units, we have a female when e is more than $+20$ and a male when e is less than -20. We expressed this relation in the following diagram for the values of e, where femaleness stands to the right of $+20$ and maleness to the left of -20. And the range between these values is the range of intersexuality. (For details see the former publications.)

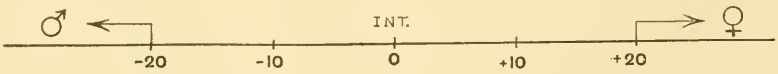

Figure 1

The experimental results showed, further, that since segregation follows the distribution of the x-chromosomes, the factor M

is carried by the latter. Further, it was proved that [FF] is inherited purely maternally and is, therefore, contained in every egg, presumably in its cytoplasm.

II

In using this modified Mendelian explanation we must keep in mind what its real meaning is. When we use Mendelian formulae we are expressing the fact that the unknown things, for which the Mendelian symbols stand, behave as units in inheritance and are distributed among the offspring of an organism unchanged and according to definite laws. When we talk about sex-factors, therefore, we only express the view that the sexual differences are due to different combinations of things of the same type as those which stand for genetic differences of other kinds, things which we symbolize as unit-factors. The mechanism of distribution of these things can again be symbolized by an imaginary system of segregation, coupling, and repulsion. Or, we understand it as the consequence of the visible distribution of definite chromosomes, thus substituting reality for symbolism. But this applies, of course, only to the mechanism of distribution of the symbolic factors and says nothing about their quality.

We are now forced to ascribe one definite quality to these symbolic factors in order to be able to explain the otherwise inexplicable results of our experiments. We recognized and expressed, from the very beginning of our work, the view that these factors have an effect of a definite quantity. And this must certainly be regarded as a first step toward replacing a symbolistic representation—and the conception of potency is again a symbolistic attribute of the symbolic factor, but one with the important quality of quantitative action—by a sound physiological conception. And now the question arises whether the results of our experiments do not enable us to replace our symbolistic interpretation by a physiological one, to give a real meaning to the words sex-factor, potency, intersexuality, the value of e, etc. The purpose of this paper is to show that we have now reached this point.

111

In studying the intersexual individuals of the different types which we bred, we realized from the very beginning the strange correlation, or rather lack of correlation, in regard to the intersexuality of the different organs. The idea of intersexuality, something between the two sexes, leads to the expectation that an intersexual individual should represent in every organ a definite intermediate step between the two sexes, equally distant from the endpoint for every organ. But this is not the case. In low intersexuality some organs are intersexual and others are not. In higher grades the hitherto unchanged organs follow and become a little intersexual, whereas those which first changed exhibit a higher grade of intersexuality. In still higher intersexuality the latter may have already assumed completely the characters of the other sex, while the others are still highly intersexual. Intersexuality is, therefore, so to speak, a macroscopic phenomenon. In reality, an intersexual animal of the per inspectionem type is a mosaic of organs of different determination in regard to their sexuality. This very important fact will be seen in the photographs of almost complete series of female and male intersexuality in figures 4 to 35, as well as in the descriptions and illustrations in our earlier papers. (A normal female and male for comparison are shown in figures 18 and 19.) The series of female intersexuality[2] should begin with an animal which is externally indistinguishable from the one shown in figure 4. The internal difference is that it is completely fertile whereas the animal pictured here is already sterile, owing to the closing of the female sex-opening. The first visible sign of intersexuality—not well shown in the picture—is that the antennae have become slightly feathered. The series for the antennae goes as far as figure 13 where the antennae are practically male. The next organ which is affected is the wing-color. In the animal of figure 5 minute streaks of male, brown pigment already appear along some veins on the left wings, and this phenomenon now increases in the series until the male

[2] There are two different series of female intersexuality, as has been communicated in former publications. This one has not been published before.

stage is finally reached. The next organ to become involved is the copulatory organ—details will be discussed later—which starts intersexuality with the animal in figure 6 and reaches practically complete maleness with the end of the series. Then follows the number of ripe eggs in the abdomen, decreasing from some hundreds to a few—the individuals in figures 11 to 15. Then follow color and hair of the abdomen, beginning to be more or less male with the animal in figure 11. Then follows the shape of the abdomen which is practically male with the animal in figure 15. Only then does the size of the abdomen begin to change, finally becoming male. But the sex-gland is—apart from details, which will be mentioned later—still female. And only in animals of the type of the last one (fig. 19) is the gradual transition of the ovary into the testis observed.

Very similar is the series of male intersexuality (figs. 20 to 33), which explains itself. It is to be noted that the female type of abdomen begins with the animal in figure 28. The last of the series (fig. 33) had a practically female abdomen. And it might be added that the change of the instincts begins in the female series with the animals in figures 9 to 11, the four last ones exhibiting practically male instincts. In the male series the change begins with the animal in figure 31, only the last of the series behaving practically like a female.

If we now try to formulate a rule which governs this strange seriation, as well as the seriation in the finer details of certain organs, we find the most important fact, *that this series is the inverse of the order of differentiation of these organs in development.* The last organs to differentiate in the pupa and the first to be intersexual are the branching of the antennae and the coloration of the wings. The first imaginal organ differentiated in the caterpillar and the last in the series to be changed toward the other sex is the sex-gland. And if we apply this law even to the minute parts of a single organ like the copulatory organ, we find it also to apply, as will be demonstrated later. Now this is the fact which, in connection with the others, enables us to formulate a definite physiological theory of sex-determination, which we propose first to consider and later to test with the facts.

IV

The situation which we face as the result of our experiments is the following. First, we recognized that the different effects of the same sex-factors in different combinations can be understood only by assuming a quantitatively different action; or, expressed in concrete terms, that the active substances, which we represent as factors, are present in different but typical quantities. Second, we were obliged to assume that these substances are distinct for each sex. Third, we realized that in the action of these substances a time factor is involved, which is definitely proportional to the quantities of the factorial substances. From these facts only one conclusion can at present be drawn: that the sex-factors are enzymes (or bodies with the properties of enzymes) which accelerate a reaction according to their concentration. Now we understand the homo-heterozygosis or one-x-two-x mechanism of normal sex-differentiation as well as the strange facts of intersexuality. In the fertilized egg the enzymes which govern the differentiation of the organism towards one of the two alternatives, maleness and femaleness, are both present. For the sake of convenience let us call them andrase and gynase. The mechanism of sex distribution—i.e., through the sex-chromosomes—results in the formation of two kinds of fertilized ova, differing in the relative concentration of the two enzymes. The formula FFMm means that the gynase is present in a higher concentration than the andrase, and the formula FFMM for the male that the concentration of the andrase is higher. The absolute concentrations are such that the constant concentration of the gynase (in female heterozygosis) is higher than one portion andrase and lower than two. And higher concentration results in greater rapidity of reaction and the more rapid reaction wins.

It is known that in mixtures of different enzymes, every single one reacts independently, provided that no interfering reaction-product is formed. Would this fact not lead to the conclusion that every animal ought to be a protandric or protogynic hermaphrodite? Theoretically this is certainly correct, although

normally only the first sex is realized. The experiments on intersexuality will show why this is the case. Let us suppose that the fertilized egg of the moth starts development. Growth and differentiation will proceed with a certain rapidity, given constant temperature, etc. During development a moment comes for every organ when its differentiation starts and when sexual differences are possible. At this point it must choose one of two alternatives, e.g., the scales of the wing must decide either to form pigment or to be filled with air. This decision must be brought about by the action of the dominating enzyme. And the one present in higher concentration will first succeed in furnishing the necessary amount of specific substance acting as determiner, which we may call the hormones of male or female differentiation. In normal sex-determination the relative concentrations of the andrase and the gynase are so fixed that the determination-point for the less concentrated one falls only after the completion of differentiation. The facts reported on the subject of intersexuality, show that when the less concentrated enzyme reaches that turning-point within the limits of embryonic differentiation, it really goes into action. Now one of several things may happen. Both hormones may act together, giving some intermediate sort of result; or the newcomer may take the action into its own hands—possibly because the supply of the other set of hormones is becoming smaller—and the differentiation assumes, from this moment on, the character of the other sex. The facts prove the latter to be the case.

If we should now work out the details of the production of the two types of intersexuality some difficulties would arise which would have to be explained. But as the more important part of the theory is its general aspect—whereas the details may possibly be conceived in different ways—we shall first show how the facts fit the general theory.

V

If the theory is correct, one of the expectations would be that normally every organ of the moth should develop under the exclusive influence of the andrase or the gynase. But if we are

confronted with conditions of abnormal concentrations of these enzymes and these concentrations are supposed to be responsible for what we call intersexuality, development must go on under the influence of one enzyme to a certain point and then continue under the influence of the other. Any organ which is completely determined before this turning-point will be purely, say, female, and every organ which is determined later, male, or vice versa; and an organ which is in process of determination during the entire period will start to develop with a female, and end with a male character. An organism that has developed in this way should be a mosaic of different degrees of maleness and femaleness, that is, it should exhibit organs of both sexes as well as organs exhibiting in themselves a mosaic of a second grade. At first sight this conclusion does not seem to coincide with the idea of intersexuality as meaning a condition between the sexes. But this discrepancy is only external. If we look at the animal as a whole, we really see a combination of the characters of both sexes. Macroscopically, so to speak, the animals are really between the sexes. The reason is that most of the characters which make up the external features of the animal are quantitative characters. Take, for example, the feathering of the antennae. In the female the side branches are short, in the male long. When development is at first female and becomes male only at a very late stage, these branches do not begin the male growth until the final change of sex starts, and then they have time to attain only an intermediate length. They appear 'intersexual' although they have developed as purely female up to a certain point, and from that point on as purely male organs. We may therefore use the term intersexuality, indicating a state of sexuality which is situated at a definite point between the sexes, if we regard the animal as a whole. But minute analysis will show that a given organ is either female or male, or has started development with one and ended it with the other sex.

This point is of such importance for the entire theory that we must go into a few details, although we do not intend to report our full evidence here. The most striking illustration can be

given by a study of the copulatory organs. The ninth abdominal segment of the female shows a pair of chitinous plates, called laminae, which flank the genital opening. They can be protruded by the aid of muscles which are inserted on chitinous rods, the apophyses. In front of this organ we find the bursa copulatrix, shown in figure 2 in ventral view. The male organs, as represented in both ventral and side views in the microphotographs figures 36 and 37 consist of a chitinous ring (the 9th segment) carrying orally and ventrally a blind sac, the saccus, dorsally and aborally a curved hook, the uncus, right and left a pair of forceps-like organs, the valvae; and in the median plane

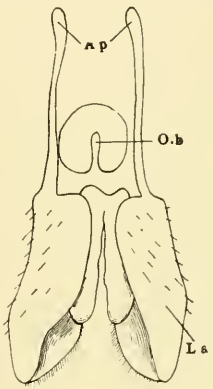

Figure 2

a long tube, the penis. The embryology of the organ shows that the first anlage, already present in the caterpillar, is the Herold's organ, from which penis and valvae later differentiate. At the time of pupation the paired anlagen of the penis are fused, and the anlagen of the valvae differentiate from the original 'Zapfen' of Herold's organ. The uncus develops in the late pupal stages and finally becomes curved. If we now study lowgrade intersexual females, the first clear indication of male parts appears in this last developing organ, the uncus, whereas the last male organs to appear in intersexuality are the first developing penis and valvae. These important facts are illustrated in the photographs 38 to 48, which represent the copulatory organs of a series of intersexual females, corresponding closely to the

series of animals pictured in figures 4 to 19. Figure 38 shows a practically female apparatus with the two laminae and the ostium bursae, the first sign of abnormality being that the distal end of the lamina is becoming pointed, which is characteristic of the uncus. This pointed condition has progressed further in figure 39, and still further in figure 40, where the laminae are well on the way toward assuming the shape of an uncus. (The asymmetrical condition of this organ is an abnormality often found in intersexual specimens.) The chitinous segment-ring, characteristic for the ninth male segment, is already in process of formation. The same is seen in figure 41 where, furthermore, the paired laminae are beginning to fuse at the base. The next step, figure 42, shows a ring-like segment with an organ unpaired proximally and paired distally, the ends being very much like an uncus. The following figure 43 shows the transforming process in progress and in figure 44 the uncus is almost complete. And only now do the first rudiments of valvae appear, figure 45, more advanced in figure 46, showing a hook in figure 47, and being almost complete in figure 48. There the penis is present, also. The intermediate stages of its formation are not figured here. (See former publications.)

A very important check for the correctness of our interpretations can now be given. In the males the first organs to develop are the valvae and penis, the last is the uncus. Therefore, when males become intersexual, we should expect the first change to appear in the uncus. This is in fact the case, as figures 49, 50, 51 show, where three types of intersexual males are represented. Everything is normal except the uncus, just becoming paired in figure 49 (viewed from the side), more so in figure 50, and beginning to assume the condition of a lamina in one of the paired pieces in figure 51.

Without going more into detail we can regard as proven the general rule: A given organ develops, in the case of female intersexuality, on female lines up to a given point, when suddenly the male stimulus starts, and the rest of the development is purely male. The degree of intersexuality is determined by how long the development has been in progress before the turning-

point occurs. For a further check we beg to compare an organ as figured in figure 44 with the pictures which Meisenheimer[3] gives of male copulatory organs, developed after extirpation of Herold's organ (from which penis and valvae derive). They are identical, the explanation being that in our case development was female and no Herold's organ was formed up to such a late point that no time for its development was left; but the rest of the development was purely male.

The importance of this set of facts makes it desirable to illustrate them further with an other organ, leaving the complete description of all details for a later monographic account. The most interesting organ is, of course, the sex-gland. We have shown in our former publications that, in the case of female intersexuality, the ovary remains up to the highest grade of intersexuality but becomes more and more rudimentary, or, rather, embryonic. And only in the highest types of female intersexuality is the ovary finally transformed into a testis. This fact agrees very well with the theory, since it is known that the sex-glands in moths are perfectly differentiated in very young caterpillars. If, therefore, the beginning of the male development does not occur at a very early[4] stage, the ovary persists, merely stopping growth and remaining embryonic. But if this turning-point arrives at a time in development when the stage of ovarian differentiation still permits a dedifferentiation, the ovary may still be transformed into a testis. We could indeed show, that in crosses which yield the latest stage of female intersexuality, young caterpillars are found with normal ovaries, whereas the adult moths show every stage of the transformation of an embryonic ovary into a testis. (The small variations, which are always found in the time of the turning-point have, of course, the result that, when development ends, some glands are completely transformed while others are not changed.)

[3] Meisenheimer, J. Experimentelle Studien zur Soma-und Geschlechtsdifferenzierung. Jena, 1909. Fig. 11, p. 21.

[4] Woltereck, R. Ueber Veraenderung der Sexualitaet bei Daphniden. Internat. Ztschr. Hydrobiol. 4, 1911. Further in Verhandlg. deutsche Zool. Ges. 1911.

In former papers we have also reported that in certain crosses where the factorial 'potencies' are extremely abnormal, nothing but males are produced, the would-be females being completely transformed into males. The theory proposed in this paper would require that in this case the turning-point is so early that, practically from the beginning of differentiation, development is male. We have recently tested the behavior of the sex-glands by killing complete cultures (all the caterpillars descended from one egg-batch, with practically no mortality) of such crosses in the five stages of the caterpillar. All these individuals in the different stages contained macroscopically and microscopically normal testes. There is only one point left unsettled. If the 'turning-point' occurs when the ovarial tubules are already well developed, the latter will cease to develop, and no more eggs will ripen. This is exactly what happens. But, if the turning-point does not occur at too late a stage, say in high-grade intersexuals, the end-filament of the ovarial tubules may possibly have time to dedifferentiate and to form testis tissue from the Ureier. An infectious disease in our cultures has prevented our testing this point, which was overlooked in former years. We hope next year to fill the gap.

To complete the evidence for the sex-glands we must see whether the theory holds good for male intersexuality. This has proved to be the case, at least as far as intersexual males have been bred (the very last steps could not be obtained). The ovary is a paired organ; the testis is paired in the caterpillar, the two parts becoming fused during pupation. In somewhat progressed intersexual males the testis is paired. Normally the testis always contains a sufficient supply of primordial sex-cells. These would be expected—if really undifferentiated—to grow into ova from the moment when the action of the gynase starts. As a matter of fact intersexual males contain in their testes nests of young ova. Thus, as far as our work has gone, the facts prove to be in perfect harmony with the theory.

VI

Starting from the foregoing facts, we might consider the theory of the sex-enzymes to be proved, in general, as completely as possible. But, if we try to work it out in detail, some difficulties appear which have to be overcome by further experimental work. The difficulty comes in at the following point. The quantitative relation of the andrase and the gynase is, in our case, such that the amount of the gynase is constant for both sexes, whereas the andrase is twice as concentrated in the male as in the female. If, now, in normal sexuality, the action of the andrase in the female falls after the end of differentiation—which, in the moth, practically coincides with hatching—the action of the gynase in the male ought to be so early that every male ought to be intersexual. This difficulty might be overcome by assuming an inactivating or retarding effect of one enzyme on the other. But this assumption would lead to new difficulties in the case of intersexuality, besides being in the nature of a loop-hole. Another way would be to ascribe to the sex-enzymes the properties of autocatalysers, which, however, also fails to work out satisfactorily in detail. A better solution is suggested by the following facts. In the gypsy-moth, as in many other insects, the male develops, under identical cultural conditions, considerably faster than the female, the difference being very regular in healthy cultures. This fact is known to every breeder because of its rather unfortunate influence upon experimental breeding, since the males are often gone by the time the females, to which they were to be mated, appear. We must suppose that this differentiating effect upon growth and metabolism is also one of the properties of the two enzymes. It is, furthermore, a fact that the same rule applies to intersexuality. We long ago observed that on an average intersexual females grow faster than normal females, and intersexual males more slowly than normal males. It becomes especially clear when, in a culture yielding nothing but males, a single female appears, or an occasional normal male in a culture yielding nothing but intersexual males. (The explanation for

these incidents will not be discussed here.) In such cases the normal individual is, in the first case, invariably the last, in the second case invariably the first, to hatch. These facts make it probable that the assumption which is illustrated in figure 3, comes near the truth. On the abscissa the time of development is marked, while the ordinate gives the progress of differentiation (and determination) during development, having reached its end—that means hatching—at the level a–a. The two full lines are then the diagrammatic time-curves of development for male and female. The vertical line (t(FF) signifies the time of

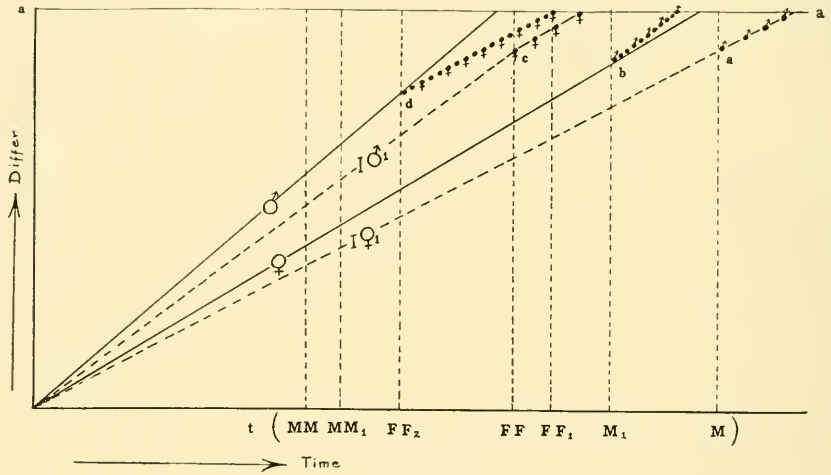

Figure 3

the initial action of FF, t(M) the same for M and t(MM) the same for MM. We see that the male has completed differentiation before crossing the line t(FF), and the female before crossing the line t(M). Female intersexuality can now be produced either by having a less concentrated FF combined with the same M, or by combining the same FF with a higher M. In the first case t(FF) would become $t(FF_1)$, lower concentration meaning a longer time of reaction. Having combined with it a slower development, the female curve would become the dotted curve I ♀ 1, which crosses the line $t(M)_1$ at the point a, that is, being a male development from this point on. In the second case, t(FF) would be left, but t(M) changed into $t(M)_1$;

the female curve crosses $t(M_1)$ at the point b and becomes the dotted line, with male determination. In the case of male intersexuality we have two similar possibilities. Either the value of MM is weakened in the hybrid combination, or the value of FF is strengthened. In the first case, the line t(MM) becomes $t(MM)_1$ and the male curve the dotted line, $I\,♂\,1$; it crosses the line t(FF) at the point c and development is female from there on. In the second case—strengthening of FF—we have the line $t(FF_2)$. The male curve cuts it at the point d, resulting in the dotted end of the curve with female determination.

This representation does away with all difficulties and has the great advantage of being open to experimental test. The tests being now under way, we shall refrain here from further discussion.

But there is one additional point which might, at least, be mentioned. In modern Mendelian discussions, the question of a possible variation of factors plays a conspicuous rôle. Most of the orthodox Mendelians decline to accept the possibility of such variation, one of them actually using the expression 'inadmissable.' We can hardly see why the assumption of the variability of a factor in regard to its quantitative value should be anything but most natural, unless we assume mystical properties for such factors. Accepting our conception of factors, there is no reason why enzymes should not exhibit slight variations in quantity, although their exact concentration seems to be one of the fundamentals of heredity. (The denomination of such variations as mutations is merely a matter of taste.) The facts which we have observed during our work are much in favor of this view. In the crosses which yield intersexual animals two types of variation can be observed. There is, first, a certain variability within a given culture (brothers and sisters). This is probably due to different conditions in development, external as well as internal, which influence the relation of the time-factor of the enzyme reaction to the progress of differentiation. The second variation concerns the results of the same cross in different individual cases. Generally this result is more or less similar, the mean of the resulting variability differing only

slightly. But occasionally one cross gives aberrant results. For example, we have made the cross of the two races which gives nothing but males some dozen times with identical results. Once only there were three extremely intersexual females, representing some minus individuals of a range of variation. This, and similar facts from other crosses, points strongly to a quantitative variation of the factor-enzymes in the gametes, the occasional extreme departures from the typical results being due to the union, in fertilization, of a minus value for one, and a plus value for the other enzyme.

VII

One of the important advances which recent genetic research has made is to furnish proof for the fact that the distribution of the chromosomes, especially the sex-chromosomes, and the symbolistic conceptions about the behaviour of Mendelian factors are one and the same thing. Thus the step from a symbolistic representation of a mechanism to the real disclosure of the mechanism has been made. We have tried in this paper to advance still further, namely, to a realization of what is moved by the mechanism and why. Do these conceptions fit the facts of cytology? We have briefly discussed that question in another place in connection with other questions, but wish again briefly to indicate the chief problem. We cannot conceive the chromosomes as built up from chromatin particles, which are themselves the chemical substratum of heredity. We believe that chromatin is a skeleton substance which works as an adsorbens for the enzymes, which really constitute the chemical basis of heredity. We have now seen how important the quantitative behavior of these enzymes is for the process of heredity. The quantity of adsorption of an enzyme by an adsorbens depends upon the qualities of both and the surface of the adsorbens. The wonderful uniformity of size and shape of the chromosomes of a given animal appears, therefore, as a minute mechanism to guarantee the typical quantity of enzymes of heredity to be assembled at the moment of fertilization. And all the strange processes preceding the maturation of the sex-cells appear

easily understandable, as well as the meaning of the peculiar mechanism of mitosis. The formation of a chromosome means, physically, the same thing as the dropping of a piece of charcoal into a solution containing enzymes.

Harrison and Doncaster[5] showed some time ago that species-crosses of the moth Biston exhibit phenomena which will probably prove to be of the same type as intersexuality in the gypsy-moth. In one of these crosses, also, exclusively males are produced. (I may take the opportunity to add here that some years ago I, also, studied species-crosses of Biston and that my results, as far as they went, agree with Harrison's experiments. However I dropped the work because of the difficulties in breeding, due to the systematic distance between the forms.) Harrison and Doncaster studied then the chromosomes of the two parental forms and found that one of the species had very much larger elements than the other. They suggested, therefore, that size of chromosomes or quantity of chromatin might be the real thing underlying our conception of different potencies. As a matter of fact we had not overlooked this possibility, and my assistant J. Seiler, had studied the chromosomes of European and Japanese gypsies. In his paper[6] which was in press when Harrison and Doncaster's was published he gives pictures of the chromosome-sets of those forms and states that the Japanese form has slightly larger chromosomes. The difference was, however, not great enough to appear very important. We have recently investigated this point again and compared the chromosome sizes of many races of known potencies. The result is not very encouraging, as nothing like a parallel between potency and chromosome size could be found. In figure 53 is given a photograph of the equatorial plate of the first maturation division in the spermatocytes of one of the forms with very high potency of the sex-factors (the Japanese race A); figure 54 represents the same stage under the same magnification from the

[5] Harrison, J. W. H. and Doncaster, L. On hybrids between moths of the geometrid subfamily Bistoninae, etc. Journ. Genetics, iii, 1914.

[6] Seiler, J. Das Verhalten der Geschlechtschromosomen bei Lepidopteren. Arch. f. Zellforsch., 13, 1914.

very weak Japanese race, H. And the chromosomes of the latter are larger. We do not, therefore, expect much information from a study of the chromosomes in our case. We believe that the adsorption of different quantities of factor-enzymes by the chromosome skeleton may sometimes be connected with visible differences of chromosome surface; but it is not at all necessary that the differences should be actually visible.

VIII

If the views which, in consequence of our experiments, we feel compelled to adopt, come near the truth, we should expect them to be applicable to other facts in regard to sex-determination (i.e., the case of Bonellia, the hormonic alteration of sex in transplantation and castration experiments in birds and mammals or in the free-martin, the case of the frog, etc.) as well as to the general facts of heredity. This is actually the case but we shall refrain from detailed discussion here. The application is so evident that it may easily be inferred. It is, moreover, by no means a new idea that factors may be regarded as enzymes. Many writers have advanced similar views, as, for example, Bateson, Guyer, Hagedoorn, Loeb, Moore, Woltereck and the writer. And Woltereck,[4] especially, has worked out the idea in regard to sex-determination, in order to explain his breeding results with Daphnids. He uses the view which we, as well as some other Mendelian writers, also used from the beginning of our work, that separate factors exist for both sexes. These factors Woltereck calls concurring sex-substances, which are present in every egg and which he conceives as zymogens. One of them can become dominant either by the action of activators or by the action of inhibitors of the alternative zymogen. Thus the zymogens are transformed into active enzymes. He then works out this conception in terms of immunochemistry. And since he needs definite cyclical changes of the relative valency and latency for the explanation of the life-cycles, he adds the necessary hypotheses for the explanation of the latter. In general his hypothesis does not differ from some Mendelian formulations which work with inhibitors; activators, changes of domi-

nance, etc., except that he speaks in terms of definite substances instead of symbolic factors. We believe that we can reach a really good explanation of the life-cycles of Daphnids by omitting some of the complications of Woltereck's hypothesis and adding to it the simple quantitative conception deduced from our work. This seems especially hopeful at present since Banta[7] has very recently communicated the discovery of intersexual strains in Daphnia. Further discussion had, therefore, better await the details of Banta's work.

Osborn Zoological Laboratory,
Yale University

[7] Banta, A. M. Sex intergrades in a species of crustacea. Proc. Nat. Acad. Sc., 2, October, 1916.

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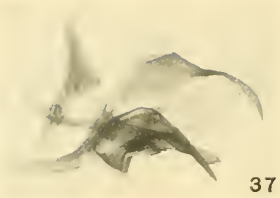

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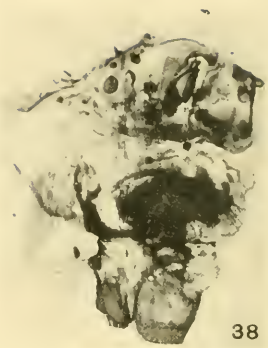

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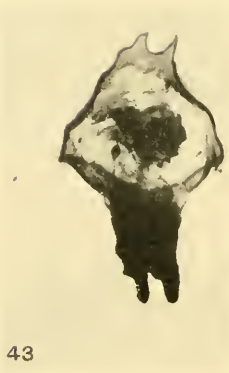

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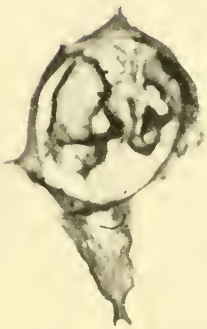

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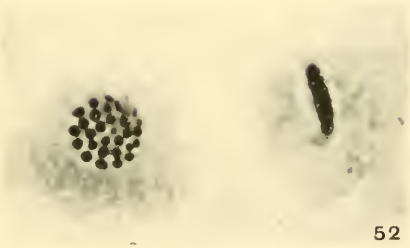

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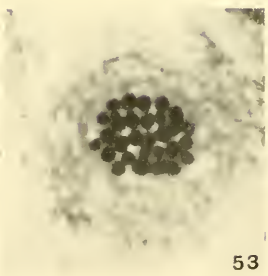

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